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Term: (monitor$$$ near5 synthesis) and (spatial$$$ near5 array)

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Search History

DATE: Thursday, June 26, 2003 Printable Copy Create Case

by their spatial addresses, and wherein the amount of the tracer moiety attached at each address is proportional to the amount of compounds attached at that address.

24. A method of making a spatially-addressable array of compounds, comprising the steps of:

(i) directly attaching, optionally by a linker, at a first address of a substrate a first compound and a first tracer moiety; and

(ii) directly attaching, optionally by a linker, a second address of a substrate a second compound and a second tracer moiety.

25. In a method of making spatially addressable array of polynucleotides by directly attaching, optionally by a linker pre-synthesized polynucleotides at a discrete spatial address on a substrate, the improvement comprising directly attaching, optionally by a linker an amount of a tracer moiety at each spatial address that is proportional to the amount of polynucleotide attached at that address.

26. In a method of making a spatially addressable array of compounds by in situ synthesis, the improvement comprising directly attaching optionally by a linker at each spatial address of the array an amount of a tracer moiety that is proportional to the amount of a product of the in situ synthesis directly attached, optionally by a linker, at that address.

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L30: Entry 17 of 18 File: USPT Jun 12, 2001

DOCUMENT-IDENTIFIER: US 6245518 B1
TITLE: Polynucleotide arrays and methods of making and using the same

Brief Summary Text (12):
In another aspect, the invention provides methods of making arrays of immobilized molecules in which each spot in the array contains an amount of a detectable label which is proportional to the amount of molecule immobilized at that spot. In the method, a molecule to be immobilized at a particular spot on the array is "spiked" with a detectable label capable of immobilizing to the substrate with the same efficiency as the molecule. The molecules to be immobilized at different spots are each "spiked" with the same proportion of label. Thus, following immobilization, each spot in the array contains an amount of label which is proportional to the efficiency of the immobilization technique. Following synthesis, the array can be scanned or otherwise analyzed for detectable signal to monitor the fidelity of the array synthesis.

Brief Summary Text (14):
In another aspect, the invention provides methods of increasing the accuracy of array-based assays. In the method, background signals produced from an array of spatially addressable immobilized molecules according to the invention are quantified and recorded.

Detailed Description Text (4):
"Spatially addressable array" refers to an array in which each element or component of the array is identifiable by its spatial address, for example its xyz coordinates. Spatial addressable arrays according to the invention can be one dimensional, for example a linear array; two dimensional; or three dimensional.

Detailed Description Text (16):
In embodiments involving immobilization of pre-synthesized polynucleotides, the polynucleotide reagent to be deposited at a particular spot contains a small quantity, typically 0.01 to 0.15%, and preferably 0.08%, of a label, typically a labeled polynucleotide. The polynucleotide reagent is then deposited on the substrate at a spatially defined region, i.e., at a particular spot. After immobilization, the spot contains an amount of labeled polynucleotide which is proportional to the amount of polynucleotide immobilized at that spot. Depositing a number of such polynucleotide reagents at different spatial addresses yields an array of polynucleotides whose sequences are identifiable by their spatial addresses. Moreover, each spot in the array contains an amount of labeled polynucleotide that is proportional to the amount of polynucleotide immobilized at that spot.

Detailed Description Text (17):
In embodiments involving in situ synthesis of polynucleotides, the polynucleotides are synthesized in their usual manner. At the synthetic step which adds the last nucleotide, the nucleoside phosphoramidite reagent to be deposited contains a small quantity, typically 0.01 to 0.15%, and preferably 0.08%, of a label, typically a labeled nucleoside phosphoramidite. The synthetic scheme yields an array of polynucleotides whose sequences are identifiable by their spatial addresses. Moreover each spot in the array contains an amount of labeled polynucleotide that is proportional to the amount of full-length polynucleotide synthesized at that spot.

CLAIMS:

1. A spatially-addressable array of compounds, comprising a substrate having directly attached thereon, optionally by a linker at each of a plurality of distinct addresses a compound and a tracer moiety, wherein the structures of the compounds are identifiable

Set Name side by side	**Query**	**Hit Count**	**Set Name** result set
	DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=NO; OP=OR		
L30	(monitor$$$ near5 synthesis) and (spatial$$$ near5 array)	18	L30
L29	(monitor$$$ near5 synthesis) and l27	4	L29
L28	l27 and monitor$$$	60	L28
L27	L26 and l21	68	L27
L26	(quality near2 control) and (polymer adj synthesis)	128	L26
L25	L24 and l21	25	L25
L24	(quality near2 control) near10 (polymer adj synthesis)	30	L24
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L22	(quality near5 control) near10 (polymer adj synthesis)	30	L22
L21	(high near5 volume) or (large near5 scale)	210042	L21
L20	(quality near5 control)	59500	L20
L19	(polymer$ near10 synthesis near10 array near10 monitor$$$$)	1	L19
L18	polymer$ near10 synthesis near10 array near10 monitor$$$$	1	L18
L17	L16 and xiang	27	L17
L16	5985356	110	L16
L15	5935856	2	L15
L14	(array near5 synthesis near10 efficiency)	14	L14
L13	(monitor$$$ near5 array near5 synthesis)	11	L13
L12	L11 with array	19	L12
L11	(efficiency near10 synthesis)	2865	L11
L10	(effciency near10 synthesis)	0	L10
L9	L7 and (cleav$$$ adj linker)	0	L9
L8	L7 and (cleav$$$ adk linker)	9	L8
L7	L6 near10 (solid adj substrate)	16	L7
L6	spatial$$$ near3 array	4662	L6
L5	l2 with efficiency	15	L5
L4	L3 and l2	10	L4
L3	indicator same efficiency	3086	L3
L2	array near5 synthesis	1424	L2
L1	array near5 syntheis	0	L1

END OF SEARCH HISTORY

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L40: Entry 39 of 41 File: USPT Dec 5, 1995

DOCUMENT-IDENTIFIER: US 5472672 A
TITLE: Apparatus and method for polymer synthesis using arrays

Brief Summary Text (21):
It is a further object of the present invention to provide a polymer array synthesis apparatus and method which is durable, compact, easy to maintain, has a minimum number of components, is easy to use by unskilled personnel, and is economical to manufacture.

Drawing Description Text (3):
FIG. 1 is an exploded top perspective view of the polymer synthesis array apparatus constructed in accordance with the present invention.

Drawing Description Text (4):
FIG. 2 is bottom perspective view of a head assembly of the polymer synthesis array apparatus of FIG. 1 illustrating the recessed nozzle ends.

Drawing Description Text (5):
FIG. 3 is a side elevation view, in cross-section of the polymer synthesis array apparatus of FIG. 1 and showing the sweeping action of the flow of inert gas through the common chamber.

Drawing Description Text (6):
FIG. 4 is a front elevation view, in cross-section of the polymer synthesis array apparatus of FIG. 1 and illustrating the head assembly pivotally mounted to a frame assembly.

Drawing Description Text (7):
FIG. 5 is an enlarged side elevation view, in cross-section, of the polymer synthesis array apparatus taken substantially along the line 5--5 of FIG. 3 and showing the capillary liquid seal formed between the liquid reagent solution and the corresponding frit and orifice.

Drawing Description Text (8):
FIG. 6 is an enlarged side elevation view, in cross-section, of the polymer synthesis array apparatus taken substantially along the line 6--6 of FIG. 3 and illustrating the balloon seal gasket.

Drawing Description Text (9):
FIG. 7 is an enlarged, schematic, top perspective view of a delivery assembly mounted to the head assembly of the polymer synthesis array apparatus.

Detailed Description Text (56):
The machine was then switched into AUTOmatic mode in order to commence actual synthesis. The machine paused during the first, second and last cycles in order to allow collection of the DEBLOCK trityl products for colorimetric analysis. This was accomplished by sliding a second 96-well microtiter plate into the lower vacuum/drain chamber, immediately beneath the capillary exit tips of the synthesis plate. The optical density of detritylation solution at 490 nanometers was read using a plate reader. The value for the first cycle confirmed the amount of CPG loaded into a well, and comparison of the second cycle with the last cycle permitted calculation of the synthesis coupling efficiency. The final cycle value also indicated the amount of oligonucleotide which will be obtained after sidechain deprotection by ammonia cleavage.

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Search Results - Record(s) 7 through 11 of 11 returned.

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7. Document ID: US 5643738 A

L13: Entry 7 of 11 File: USPT Jul 1, 1997

US-PAT-NO: 5643738
DOCUMENT-IDENTIFIER: US 5643738 A

TITLE: Method of synthesis of plurality of compounds in parallel using a partitioned solid support

DATE-ISSUED: July 1, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zanzucchi; Peter John	West Windsor Township, Mercer County	NJ		
Cherukuri; Satyam Choudary	Cranbury	NJ		
McBride; Sterling Edward	Lawrence Township, Mercer County	NJ		
Judd; Amrit Kaur	Belmont	CA		

US-CL-CURRENT: 435/6; 422/131, 422/134, 525/54.11, 536/25.3, 536/25.31

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

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8. Document ID: US 5593838 A

L13: Entry 8 of 11 File: USPT Jan 14, 1997

US-PAT-NO: 5593838
DOCUMENT-IDENTIFIER: US 5593838 A

TITLE: Partitioned microelectronic device array

DATE-ISSUED: January 14, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zanzucchi; Peter J.	West Windsor Township	NJ		
Cherukuri; Satyam C.	Cranbury	NJ		
McBride; Sterling E.	Lawrence Township	NJ		
Judd; Amrit K.	Belmont	CA		

US-CL-CURRENT: 435/6; 204/450, 435/287.2, 435/288.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

9. Document ID: US 5585069 A

L13: Entry 9 of 11 File: USPT Dec 17, 1996

US-PAT-NO: 5585069
DOCUMENT-IDENTIFIER: US 5585069 A

TITLE: Partitioned microelectronic and fluidic device array for clinical diagnostics and chemical synthesis

DATE-ISSUED: December 17, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zanzucchi; Peter J.	Lawrenceville	NJ		
Cherukuri; Satyam C.	Cranbury	NJ		
McBride; Sterling E.	Lawrenceville	NJ		

US-CL-CURRENT: 422/100; 204/450, 204/600, 422/58, 422/68.1, 436/43

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC
Draw Desc | Image

10. Document ID: JP 06069724 A

L13: Entry 10 of 11 File: JPAB Mar 11, 1994

PUB-NO: JP406069724A
DOCUMENT-IDENTIFIER: JP 06069724 A
TITLE: ARRAY ANTENNA

PUBN-DATE: March 11, 1994

INVENTOR-INFORMATION:

NAME	COUNTRY
TERAUCHI, MUTSUE	
SATO, MITSUHISA	

INT-CL (IPC): H01Q 21/06

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Clip Img | Image

11. Document ID: JP 11234036 A

L13: Entry 11 of 11 File: DWPI Aug 27, 1999

DERWENT-ACC-NO: 1999-535297
DERWENT-WEEK: 199945

TITLE: Beam synthesis procedure of antenna array for use in millimeter waveband for e.g. in vehicle monitoring radar, high speed wireless base station - involves setting length and period of wave leakage portion of each antenna unit in accord with radiation

angle and intensity of N-divided points on specific end portions of antenna units to be combined

PRIORITY-DATA: 1998JP-0052755 (February 18, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 11234036 A	August 27, 1999		010	H01Q021/06

INT-CL (IPC): H01 P 5/02; H01 P 5/12; H01 Q 1/48; H01 Q 13/20; H01 Q 19/28; H01 Q 21/06

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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Terms	Documents
(monitor$$$ near5 array near5 synthesis)	11

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16584 LIBRAR#####
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510573 MULTIPL?
102134 COLLECTION#
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140129 RANDOM
160132 MOLECULE#
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87713 CHEMIST#####
345490 SYNTHE#####
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OR
SYNTHE##### OR COMBINATORIAL)

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345490 SYNTHE#####
143922 MATRIX
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699212 SUPPORT
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AY)

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256548 MONITOR###
125726 SYNTHES###
440 MONITOR### (4A) SYNTHES###
L3 44 L2 AND (MONITOR### (4A) SYNTHES###)

=> s l2 and (monitor### (3a) synthes###)

256548 MONITOR###
125726 SYNTHES###
380 MONITOR### (3A) SYNTHES###
L4 35 L2 AND (MONITOR### (3A) SYNTHES###)

=> d l4 1-5

1. 5,736,336, Apr. 7, 1998, Peptide nucleic acids having enhanced binding affinity, sequence specificity and solubility; Ole Buchardt, deceased, et al., 435/6; 436/501; 530/300, 333, 350; 536/23.1, 24.1, 24.3, 24.31, 24.32, 24.33, 25.3; 935/77, 78 [IMAGE AVAILABLE]

2. 5,736,336, Apr. 7, 1998, Peptide nucleic acids having enhanced binding affinity, sequence specificity and solubility; Ole Buchardt, deceased, et al., 435/6; 436/501; 530/300, 333, 350; 536/23.1, 24.1, 24.3, 24.31, 24.32, 24.33, 25.3; 935/77, 78 [IMAGE AVAILABLE]

3. 5,719,262, Feb. 17, 1998, Peptide nucleic acids having amino acid side chains; Ole Buchardt, deceased, et al., 530/300; 435/6; 436/501; 530/350; 536/23.1, 24.1, 24.3, 24.31, 24.32, 24.33; 935/77, 78 [IMAGE AVAILABLE]

4. 5,719,060, Feb. 17, 1998, Method and apparatus for desorption and ionization of analytes; T. William Hutchens, et al., 436/174; 250/287, 288; 436/63, 173, 178 [IMAGE AVAILABLE]

5. 5,716,785, Feb. 10, 1998, Processes for genetic manipulations using promoters; Russell N. Van Gelder, et al., 435/6, 91.21; 536/23.1, 24.1, 25.3 [IMAGE AVAILABLE]

=> d 1 cit ab kwic

1. 5,736,336, Apr. 7, 1998, Peptide nucleic acids having enhanced binding affinity, sequence specificity and solubility; Ole Buchardt, deceased, et al., 435/6; 436/501; 530/300, 333, 350; 536/23.1, 24.1, 24.3, 24.31, 24.32, 24.33, 25.3; 935/77, 78 [IMAGE AVAILABLE]

US PAT NO: 5,736,336 [IMAGE AVAILABLE] L4: 1 of 35

ABSTRACT:
A novel class of compounds, known as peptide nucleic acids, bind complementary DNA and RNA strands more strongly than a corresponding DNA strand, and exhibit increased sequence specificity and solubility. The peptide nucleic acids comprise ligands selected from a group consisting of naturally-occurring nucleobases and non-naturally-occurring nucleobases attached to a polyamide backbone, and contain C.sub.1 -C.sub.8 alkylamine side chains. Methods of enhancing the solubility, binding affinity and sequence specificity of PNAs are provided.

SUMMARY:

BSUM(31)

The PNAs of the invention are **synthesized** by adaptation of standard peptide **synthesis** procedures, either in solution or on a **solid** phase.

DRAWING DESC:

DRWD(4)

FIG. 3 provides a general scheme for **solid** phase PNA **synthesis** illustrating the preparation of linear unprotected PNA amides.

DETDESC:

DETD(7)

The . . . invention may also be attached to water-soluble polymer, water-insoluble polymers, oligonucleotides or carbohydrates. When warranted, a PNA oligomer may be **synthesized** onto a moiety (e.g., a peptide chain, reporter, intercalator or other type of ligand-containing group) attached to a **solid** **support**.

DETDESC:

DETD(14)

The principle of anchoring molecules during a reaction onto a **solid** **matrix** is known as **Solid** Phase **Synthesis** or Merrifield **Synthesis** (see Merrifield, J. Am. Chem. Soc., 1963, 85, 2149 and Science, 1986, 232, 341). Established methods for the stepwise or fragment-wise **solid** phase assembly of amino acids into peptides normally employ a beaded **matrix** of cross-linked styrene-divinylbenzene copolymer. The cross-linked copolymer is formed by the pearl polymerization of styrene monomer to which is added a mixture of divinylbenzenes. Usually, 1-2% cross-linking is employed. Such a **matrix** may be used in **solid** phase PNA **synthesis** of the present invention (FIG. 3).

DETDESC:

DETD(15)

More than fifty methods for initial functionalization of the **solid** phase have been described in connection with traditional **solid** phase peptide **synthesis** (see Barany and Merrifield in "The Peptides" Vol. 2, Academic Press, New York, 1979, pp. 1-284, and Stewart and Young, "**Solid** Phase Peptide **Synthesis**", 2nd Ed., Pierce Chemical

Company, Illinois, 1984). Reactions for the introduction of chloromethyl functionality (Merrifield resin; via a chloromethyl methyl. . . Chem. Soc., 1970, 650) are most widely used. Regardless of its nature, the purpose of introducing a functionality on the **solid** phase is to form an anchoring linkage between the copolymer **solid** **support** and the C-terminus of the first amino acid to be coupled to the **solid** **support**. As will be recognized, anchoring linkages may also be formed between the **solid** **support** and the amino acid N-terminus. The "concentration" of a functional group present in the **solid** phase is generally expressed in millimoles per gram (mmol/g). Other reactive functionalities which have been initially introduced include 4-methylbenzhydrylamino and. . .

DETDESC:

DETD(17)

Certain functionalities (e.g., benzhydrylamino, 4-methylbenzhydrylamino and 4-methoxybenzhydrylamino), which may be incorporated for the purpose of cleavage of a **synthesized** PNA chain from the **solid** **support** such that the C-terminal of the PNA chain is released as an amide, require no introduction of a spacer group.. . .

DETDESC:

DETD(18)

An alternative strategy concerning the introduction of spacer or handle groups is the so-called "preformed handle" strategy (see Tam et al., **Synthesis**, 1979, 955-957), which offers complete control over coupling of the first amino acid and excludes the possibility of complications arising from the presence of undesired functional groups not related to the peptide or PNA **synthesis**. In this strategy, spacer or handle groups, of the same type as described above, are reacted with the first amino acid desired to be bound to the **solid** **support**, the amino acid being N-protected and optionally protected at the other side chains which are not relevant with respect to. . . those cases in which a spacer or handle group is desirable, the first amino acid to be coupled to the **solid** **support** can either be coupled to the flee reactive end of a spacer group which has been bound to the initially. . . J. Org. Chem., 1985, 50, 5291), which provide more than one mode of release and thereby allow more flexibility in **synthetic** design.

DETDESC:

DETD(22)

Following coupling of the first amino acid, the next stage of **solid** phase **synthesis** is the systematic elaboration of the desired PNA chain. This elaboration involves repeated deprotection/coupling cycles. A temporary protecting group, such. . .

DETDESC:

DETD(24)

Following . . . the next step will normally be deprotection of the amino acid moieties of the PNA chain and cleavage of the **synthesized** PNA from the **solid** **support**. These processes can take place substantially simultaneously, thereby providing the free PNA molecule in the desired form. Alternatively, in cases in which condensation of two separately **synthesized** PNA chains is to be carried out, it is possible, by choosing a suitable spacer group at the start of the **synthesis**, to cleave the desired PNA chains from their respective **solid** supports (both peptide chains still incorporating their side chain-protecting groups) and finally removing the side chain-protecting groups after, for example,. . .

DETDESC:

DETD(25)

In the above-mentioned "BOC-benzyl" protection scheme, the final deprotection of side chains and release of the PNA molecule from the **solid** **support** is most often carried out by the use of strong acids such as anhydrous HF (Sakakibara et al., Bull. Chem.. . . method, which removes the precursors of harmful carbocations to form inert sulfonium salts, is frequently employed in peptide and PNA **synthesis**. Other methods for deprotection and/or final cleavage of the PNA-**solid** **support** bond may include base-catalyzed alcoholysis (Barton et al., J. Am. Chem. Soc., 1973, 95, 4501), ammonolysis, hydrazinolysis (Bhydrazinolysis (Bodanszky et. . .

DETDESC:

DETD(27)

Based on the recognition that most operations are identical in the **synthetic** cycles of **solid** phase peptide **synthesis** (as is also the case for **solid** phase PNA **synthesis**), a new **matrix**, PEPS, was recently introduced (Berg et al., J. Am. Chem. Soc., 1989, 111, 8024 and International Patent Application WO 90/02749) to facilitate the preparation of a large number of peptides. This **matrix** is comprised of a polyethylene (PE) film with pendant long-chain polystyrene (PS) grafts (molecular weight on the order of 10.sup.6 Daltons). The loading capacity of the film is as high as that of a beaded **matrix**, but PEPS has the additional flexibility to suit **multiple** **syntheses** simultaneously. Thus, in a new configuration for **solid** phase peptide **synthesis**, the PEPS film is fashioned in the form of discrete, labeled sheets, each serving as an individual compartment. During all the identical steps of the **synthetic** cycles, the sheets are kept together in a single reaction vessel to permit concurrent preparation of a multitude of peptides at a rate close to that of a single peptide **synthesis** by conventional methods. It is believed that the PEPS film **support**, comprising linker or spacer groups adapted to the particular chemistry will be particularly valuable in the **synthesis** of **multiple** PNA **molecules**. The **synthesis** of PNAs are conceptually simple because only four different reaction compartments are normally required, one for each of the four "pseudo-nucleotide" units. The PEPS film **support** has been successfully tested in a number of PNA **syntheses** carried out in a parallel and substantially simultaneous fashion. The yield and quality of the products obtained from PEPS are comparable to those obtained by using the traditional polystyrene bead **support**. Also, experiments with other geometries of the PEPS polymer, for example, non-woven felt, knitted net, sticks and microwellplates, have not indicated any limitations of the **synthetic** efficacy.

DETDESC:

DETD(28)

Two other methods for the simultaneous **synthesis** of large numbers of peptides also apply to the preparation of **multiple**, different PNA **molecules**. The first of these methods (Geysen et al., Proc. Natl. Acad. Sci. USA, 1984, 81, 3998) utilizes acrylic acid-grafted polyethylene-rods and 96-microtiter wells to immobilize the growing peptide chains and to perform the compartmentalized **synthesis**. While effective, this method is only applicable on a microgram scale. The second method (Houghten, Proc. Natl. Acad. Sci. USA, 1985, 82, 5131) utilizes a "tea bag" containing traditionally-used polymer beads. Other methods for **multiple** peptide or PNA **synthesis** in the context of the present invention include the simultaneous us- -f two different supports with different densities (Tregear in. . . Sci. Publ., Ann Arbor, 1972, pp. 175-178), combining reaction vessels via a manifold (Gorman, Anal. Biochem., 1984, 136, 397), multicolumn **solid** phase **synthesis** (Krchnak et al., Int. J. Peptide Protein Res., 1989, 33, 209, and Holm and Meldal in "Proceedings of the 20th. . .

DETDESC:

DETD(29)

Conventional cross-linked styrene/divinylbenzene copolymer **matrix** and the PEPS **support** are preferred in the context of **solid** phase PNA **synthesis**. Other exemplary **solid** supports include (1) particles based upon copolymers of dimethylacrylamide cross-linked with N,N'-bisacryloylethylenediamine, (2) **solid** supports based on silica-containing particles such as porous glass beads and silica gel, (3) composites that contain two major ingredients:. . . and van Rietschoten in "Peptides 1974", Y. Wolman, Ed., Wiley and Sons, New York, 1975, pp. 113-116) and (4) contiguous **solid** supports other than PEPS, such as cotton sheets (Lebl and Eichler, Peptide Res., 1989, 2, 232) and hydroxypropylacrylate-coated polypropylene membranes. . .

DETDESC:

DETD(30)

Whether manually or automatically operated, **solid** phase PNA **synthesis**, in the context of the present invention, is normally performed batchwise. However, most of the **syntheses** may be carried out equally well in the continuous-flow mode, where the **support** is packed into columns (Bayer et al., Tetrahedron Lett., 1970, 4503; and Scott et al., J. Chromatogr. Sci., 1971, 9, 577). With respect to continuous-flow **solid** phase **synthesis**, the rigid poly(dimethylacrylamide)-Kieselguhr **support** (Atherton et al., J. Chem. Soc. Chem. Commun., 1981, 1151) appears to be particularly useful. Another useful configuration is the one worked out for the standard copoly(styrene-1%-divinylbenzene) **support** (Krchnak et al., Tetrahedron Lett., 1987, 4469).

DETDESC:

DETD(31)

While the **solid** phase technique is preferred in the present invention, other methodologies or combinations thereof may also be used. Exemplary methodologies include (1) the classical solution phase methods for peptide **synthesis** (Bodanszky, "Principles of Peptide **Synthesis**", Springer-Verlag, Berlin-New York, 1984), either by step-wise assembly or by segment/fragment condensation, (2) the "liquid phase" strategy, which utilizes soluble. . . amino acid active esters (Fridkin et al., J. Am. Chem. Soc., 1965, 87, 4646), sometimes referred to as "inverse Merrifield **synthesis**" or "polymeric reagent **synthesis**". In addition, it is envisaged that PNA molecules may be assembled enzymatically by enzymes such as proteases or derivatives thereof. . . Finally, completely artificial enzymes, very recently pioneered by Hahn et al. (Science, 1990, 248, 1544), may be developed for PNA **synthesis**. The design of generally applicable enzymes, ligases, and catalytic antibodies, capable of mediating specific coupling reactions, should be more readily achieved for PNA **synthesis** than for "normal" peptide **synthesis** since PNA molecules will often be comprised of only four different amino acids (one for each of the four native. . .

DETDESC:

DETD(40)

The present invention also pertains to the advantageous use of PNA molecules in **solid** phase biochemistry (see "**Solid** Phase Biochemistry--Analytical and **Synthetic** Aspects", W. H. Scouten, Ed., John Wiley & Sons, New York, 1983), notably **solid** phase biosystems, especially bioassays or **solid** phase techniques for diagnostic detection/quantitation or affinity purification of complementary nucleic acids (see "Affinity Chromatography--A Practical Approach", P. D. G.. . . utilize "normal" or slightly modified oligonucleotides either physically adsorbed or bound through a substantially permanent covalent

anchoring linkage to beaded **solid** supports such as cellulose, glass beads, including those with controlled porosity (Mizutani et al., J. Chromatogr., 1986, 356, 202), "Sephadex",. . .

DETDESC:

DETD(43)

As for conventional **solid** phase peptide **synthesis**, however, the latter supports are excellent materials for building up immobilized PNA molecules. It allows the side chain-protecting groups to be removed from the **synthesized** PNA chain without cleaving the anchoring linkage holding the chain to the **solid** **support**. They also can be loaded onto **solid** supports in large amounts, thus further increasing the capacity of the **solid** phase technique. Furthermore, certain types of studies concerning the use of PNA in **solid** phase biochemistry can be conducted, facilitated, or greatly accelerated by use of the recently-reported "light-directed, spatially addressable, parallel chemical **synthesis**" technology (Fodor et al., Science, 1991, 251, 767), a technique that combines **solid** phase chemistry and photolithography to produce thousands of highly diverse, but identifiable, permanently immobilized compounds (such as peptides) in a. . .

DETDESC:

DETD(50)

The . . . t-butoxycarbonyl; Z, benzyloxycarbonyl; NMR, nuclear magnetic resonance; s, singlet; d, doublet; dd, doublet of doublets; t; triplet; q, quartet; m, **multiplet**; b, broad; .delta., **chemical** shift; ppm, parts per million (chemical shift).

DETDESC:

DETD(61)

This procedure is different from the literature **synthesis**, but is easier, gives higher yields, and leaves no unreacted thymine in the product. To a suspension of thymine (3,. . . mmol). The mixture was stirred vigorously overnight under nitrogen. The mixure was filtered and evaporated to dryness, in vacuo. The **solid** residue was treated with water (300 mL) and 4N hydrochloric acid (12 mL), stirred for 15 minutes at 0.degree. C.,. . .

DETDESC:

DETD(104)

Except where otherwise stated, the following applies. The PNA compounds were **synthezised** by the stepwise **solid** phase approach (Merrifield, J. Am. Chem. Soc., 1963, 85, 2149) employing conventional peptide chemistry utilizing the TFA-labile tert-butyloxycarbonyl (BOC) group. . . resin (Matsueda et al., Peptides, 1981, 2, 45). All reactions (except HF reactions) were carried out in manually operated standard **solid** phase reaction vessels fitted with a coarse glass frit (Merrifield et al., Biochemistry, 1982, 21, 5020). The quantitative ninhydrin reaction. . . n (assuming both complete deprotection and coupling as well as neither chain termination nor loss of PNA chains during the **synthetic** cycle) is calculated from the equation:

DETDESC:

DETD(108)

Solid Phase **Synthesis** of Acr.sup.1 -[Taeg].sub.15 -NH.sub.2 and Shorter Derivatives

DETDESC:

DETD(110)

The **synthesis** was initiated on 100 mg of preswollen and neutralized BHA resin (determined by the quantitative ninhydrin reaction to contain 0.57 mmol NH.sub.2 /g) employing single couplings ("**Synthetic** Protocol 1") using 3.2 equivalents of BOC-Taeg-OPfp in about 33% DMF/CH.sub.2 Cl.sub.2. The individual coupling reactions were carried out by shaking for at least 12 h in a manually operated 6 mL standard **solid** phase reaction vessel and unreacted amino groups were blocked by acetylation at selected stages of the **synthesis**. The progress of chain elongation was monitored at several stages by the quantitative ninhydrin reaction (see Table I). Portions of. . .

DETDESC:

DETD(125)

Solid Phase **Synthesis** of Acr.sup.1 -[Taeg].sub.15 -Lys-NH.sub.2 and Shorter Derivatives

DETDESC:

DETD(127)

The **synthesis** was initiated by a quantitative loading (standard DCC in situ coupling in neat CH.sub.2 Cl.sub.2) of BOC-Lys(ClZ) onto 100 mg. . . of preswollen and neutralized BHA resin (0.57 mmol NH.sub.2 /g). Further extension of the protected PNA chain employed single couplings ("**Synthetic** Protocol 2") for cycles 1 to 5 and cycles 10 to 15 using 3.2 equivalents of BOC-Taeg-OPfp in about 33%. . . All coupling reactions were carried out by shaking for at least 12 h in a manually operated 6 mL standard **solid** phase reaction vessel. Unreacted amino groups were blocked by acetylation at the same stages of the **synthesis** as was done in Example 17. Portions of protected BOC-[Taeg].sub.5 -Lys(ClZ)-BHA and BOC-[Taeg].sub.10 -Lys(ClZ)-BHA resins were removed after assembling 5. . . (see section (e)), an additional "free acid" coupling of PNA residues 5 to 10 gave no significant improvement of the **synthetic** yield as compared to the throughout single-coupled residues in Example 17.

DETDESC:

DETD(143)

Improved **Solid** Phase **Synthesis** of H-[Taeg].sub.10 -Lys-NH.sub.2

DETDESC:

DETD(150)

About . . . growing chains, taken out of totally .about. 19 g wet resin prepared in section (b)) was placed in a 55 mL **solid** phase peptide **synthesis** (SPPS) reaction vessel. BOC-[Taeg].sub.8 -Lys(ClZ)-MBHA resin was assembled by single couplings ("**Synthetic** Protocol 4") utilizing 2.5 equivalents of BOC-Taeg-OPfp in about 30% DMF/CH.sub.2 Cl.sub.2. The progress of the **synthesis** was **monitored** at all stages by the quantitative ninhydrin reaction (see Table II).

DETDESC:

DETD(152)

About . . . section utilizing 2.5 equivalents of BOC-Taeg-OPfp in about 30% DMF/CH.sub.2 Cl.sub.2. The reaction volume was 3 mL (vigorous shaking). The **synthesis** was **monitored** by the quantitative ninhydrin reaction (see Table II).

DETDESC:

DETD(264)

Solid Phase **Synthesis** of H-[Taeg].sub.5 -[Gaeg]-[Taeg].sub.4 -Lys-NH.sub.2

DETDESC:

DETD(269)

Solid Phase **Synthesis** of H-Taeg-Aaeg-[Taeg].sub.8 -Lys-NH.sub.2

DETDESC:

DETD(271)

About . . . mL of 50% DMF/CH.sub.2 Cl.sub.2 and a single coupling with 0.15M BOC-Taeg-OPfp in neat CH.sub.2 Cl.sub.2 ("Synthetic Protocol 5"). The **synthesis** was **monitored** by the quantitative ninhydrin reaction, which showed about 50% incorporation of A(Z)aeg and about 96% incorporation of Taeg.

DETDESC:

DETD(277)

Solid Phase **Synthesis** of H-[Taeg].sub.2 -Aaeg-[Taeg].sub.5 -Lys-NH.sub.2

DETDESC:

DETD(279)

About . . . monomer (free acid) together with an equivalent amount of DCC in 2 mL neat CH.sub.2 Cl.sub.2 ("Synthetic Protocol 6"). The **synthesis** was **monitored** by the quantitative ninhydrin reaction which showed a total of about 82% incorporation of A(Z)aeg after coupling three times (the. . .

DETDESC:

DETD(303)

Solid Phase **Synthesis** of H-[Taeg].sub.2 -Aaeg-Taeg-Caeg-Aaeg-Taeg-Caeg-Taeg-Caeg-Lys-NH2

DETDESC:

DETD(305)

About . . . the five last residues. Each coupling reaction was allowed to proceed for a total of 20-24 h with shaking. The **synthesis** was **monitored** by the ninhydrin reaction, which showed nearly quantitative incorporation of all residues except of the first A(Z)aeg residue, which had. . .

DETDESC:

DETD(309)

Solid Phase **Synthesis** of H-[Taeg].sub.5 -Lys(ClZ)-MBHA Resin

DETDESC:

DETD(322)

Solid Phase **Synthesis** of H-Tyr-[Taeg].sub.10 -Lys-NH.sub.2

DETDESC:

DETD(328)

Solid Phase **Synthesis** of Dansyl-[Taeg].sub.10 -Lys-NH.sub.2

DETDESC:

DETD(334)

Solid Phase **Synthesis** of H-[Taeg].sub.3 -Caeg-[Taeg].sub.4 -NH.sub.2

DETDESC:

DETD(336)

About . . . with 0.13M BOC-Taeg-OPfp in 2.5 mL of CH.sub.2 Cl.sub.2. Each coupling reaction was allowed to proceed with shaking overnight. The **synthesis** was **monitored** by the ninhydrin reaction, which showed close to quantitative incorporation of all the residues.

DETDESC:

DETD(340)

Solid Phase **Synthesis** of H-[Taeg].sub.2 -Caeg-[Taeg].sub.2 -Caeg-[Taeg].sub.4 -Lys-NH.sub.2

DETDESC:

DETD(425)

The **solid** **support** (BOC-BHA-PEG-resin) is washed with 708 .mu.l of Wash A. Deblock (177 .mu.L) is passed through the column 3 times over. . . Cap solution over 5 minutes. The resin is then washed 2124 .mu.L of Wash A. The cycle is repeated until **synthesis** of the desired PNA sequence is completed.

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(FILE 'USPAT' ENTERED AT 12:25:23 ON 14 APR 1998)

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113286 LABEL####

256602 MONITOR######

5004 LABEL#### (P)MONITOR######

L5 7 L3 AND (LABEL#### (P)MONITOR######)

=> d l5 1-5

1. 5,643,722, Jul. 1, 1997, Methods for the detection and isolation of proteins; Kenneth J. Rothschild, et al., 435/6, 7.2, 70.1, 71.1 [IMAGE AVAILABLE]

2. 5,468,481, Nov. 21, 1995, MHC class II-peptide conjugates useful in ameliorating autoimmunity; Somesh D. Sharma, et al., 424/185.1, 184.1, 193.1, 278.1; 514/2, 8; 530/395, 402, 403, 868 [IMAGE AVAILABLE]

3. 5,366,862, Nov. 22, 1994, Method for generating and screening useful peptides; Duane L. Venton, et al., 435/7.1, 23, 68.1; 436/501, 518; 530/338, 343 [IMAGE AVAILABLE]

4. 5,284,935, Feb. 8, 1994, MHC-mediated toxic conjugates useful in ameliorating autoimmunity; Brian R. Clark, et al., 424/185.1, 193.1, 810; 530/395, 403, 806, 807, 868 [IMAGE AVAILABLE]

5. 5,260,422, Nov. 9, 1993, MHC conjugates useful in ameliorating autoimmunity; Brian R. Clark, et al., 424/185.1, 193.1, 810; 530/402, 403, 868 [IMAGE AVAILABLE]

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1. 5,643,722, Jul. 1, 1997, Methods for the detection and isolation of proteins; Kenneth J. Rothschild, et al., 435/6, 7.2, 70.1, 71.1 [IMAGE AVAILABLE]

US PAT NO: 5,643,722 [IMAGE AVAILABLE] L5: 1 of 7

ABSTRACT:
The invention is directed to methods for the non-radioactive labeling, detection, quantitation and isolation of nascent proteins translated in a cellular or cell-free translation system. tRNA molecules are misaminoacylated with non-radioactive markers which may be non-native amino acids, amino acid analogs or derivatives, or substances recognized by the protein synthesizing machinery. Markers may comprise cleavable moieties, detectable labels, reporter properties wherein markers incorporated into protein can be distinguished from unincorporated markers, or coupling agents which facilitate the detection and isolation of nascent protein from other components of the translation system. The invention also comprises proteins prepared using misaminoacylated tRNAs which can be utilized in pharmaceutical compositions for the treatment of diseases and disorders in humans and other mammals, and kits which may be used for the detection of diseases and disorders.

SUMMARY:

BSUM(7)

A . . . performed outside the cell in systems referred to as cell-free translation systems. In either system, the basic process of protein **synthesis** is identical. The extra-cellular or cell-free translation system comprises an extract prepared from the intracellular contents of cells. These preparations contain those molecules which **support** protein translation and depending on the method of preparation, post-translational events such as glycosylation and cleavages as well. Typical cells. . .

SUMMARY:

BSUM(13)

The use of radioactive **labeled** amino acids also does not allow for a simple and rapid means to **monitor** the production of nascent proteins inside a cell-free extract without prior separation of nascent from preexisting proteins. However, a separation. . .

DETDESC:

DETD(13)

Misaminoacylated tRNAs are introduced into the cellular-or cell-free protein **synthesis** system. In the cell-free protein **synthesis** system, the reaction mixture contains all the cellular components necessary to **support** protein **synthesis** including ribosomes, tRNA, rRNA, spermidine and physiological ions such as magnesium and potassium at appropriate concentrations and an appropriate pH.. . .

DETDESC:

DETD(22)

Markers . . . transferred from a charged tRNA into a growing peptide chain. To be useful, markers must also possess certain physical and physio-**chemical** properties. Therefore, there are **multiple** criteria which can be used to identify a useful marker. First, a marker must be suitable for incorporation into a. . .

DETDESC:

DETD(44)

Another embodiment of the invention is directed to a method for **monitoring** the **synthesis** of nascent proteins in a cellular or a cell-free protein synthesis system without separating the components of the system. These. . .

DETDESC:

DETD(53)

As stated above, a principal advantage of using reporters is the ability to **monitor** the **synthesis** of proteins in cellular or a cell-free translation systems directly without further purification or isolation steps. Reporter markers may also. . .

2. 5,468,481, Nov. 21, 1995, MHC class II-peptide conjugates useful in ameliorating autoimmunity; Somesh D. Sharma, et al., 424/185.1, 184.1, 193.1, 278.1; 514/2, 8; 530/395, 402, 403, 868 [IMAGE AVAILABLE]

US PAT NO: 5,468,481 [IMAGE AVAILABLE] L5: 2 of 7

ABSTRACT:
The present invention is directed to complexes consisting essentially of an isolated MHC component and an autoantigenic peptide associated with the antigen binding site of the MHC component. These complexes are useful in treating autoimmune disease.

DETDESC:

DETD(40)

The Dupont apparatus and technique for rapid **multiple** peptide **synthesis** (RAMPS) is used to **synthesize** the members of a set of overlapping (10 residue overlap), 20-residue peptides from the alpha subunit of Torpedo californicus AChR.. . . this peptide is known and is shown in FIG. 6. One or more radioactive amino acids is incorporated into each **synthetic** peptide. The pentafluorphenyl active esters of side chain-protected, FMOC amino acids are used to **synthesize** the peptides, applying standard stepwise **solid** phase peptide **synthetic** methods, followed by standard side chain deprotection and simultaneous release of the peptide amide from the **solid** **support**.

DETDESC:

DETD(48)

The identified peptides are then prepared by conventional **solid** phase **synthesis** and the subset which contain epitopes for the disease-inducing helper T cell clones is determined by incubation of the candidate. . .

DETDESC:

DETD(71)

A . . . individuals T cells are examined in vitro, to determine the autopeptide(s) recognized by autoreactive T cells; this is accomplished

utilizing **labeled** complexes of the invention, described supra, which are of the formula X.sup.1 MHC.sup.2 peptide, wherein X is a **label** moiety. After it is determined which complexes target the T cells, the individual is treated with complexes of the invention. . . is a moiety capable of killing the T cell), respectively. Therapy (as determined by the autoreactive T cells remaining) is **monitored** with T cell binding studies using the **labeled** complexes of the invention, described supra.

DETDESC:

DETD(150)

The . . . in a proliferation assay. Uptake of 3-(4,5-dimethyl-thiazol-2-7)-2,5 diphenyltetrazolium bromide (MTT) was used as an indication of cell proliferation. Although DNA **synthesis**, usually **monitored** by .sup.3 H-thymidine uptake, and the activity of mitochondria, measured by MTT uptake, are different cellular functions, it has been. . .

DETDESC:

DETD(225)

Torpedo AChRe subunit peptide 100-116 (YAIVHMTKLLLDYPGKI) was **synthesized** by **solid**-phase 9-fluorenylmethoxycarbonyl (FMOC) procedures, using standard procedures. The peptides were purified by reverse-phase HPLC, and characterized by HPLC and mass spectroscopy.

3. 5,366,862, Nov. 22, 1994, Method for generating and screening useful peptides; Duane L. Venton, et al., 435/7.1, 23, 68.1; 436/501, 518; 530/338, 343 [IMAGE AVAILABLE]

US PAT NO: 5,366,862 [IMAGE AVAILABLE] L5: 3 of 7

ABSTRACT:
The invention allows the generation and screening of a large population of peptides for the presence of peptides which bind a particular macromolecule or macromolecular complex with high affinity, and further allows the favored net synthesis of analyzable quantities of such peptides, by using as the "trap" a macromolecule or macromolecular complex for which binding of the peptide is desired. The starting mixture is preferably spiked with a peptide having some affinity for the target macromolecule so that mutation of the spike or "lead" peptide is favored. The development of improved binding peptides through scrambling may be dynamically monitored by initially binding the target with an insolubilized ligand, and then looking for an increase in the concentration of the target in the soluble phase as a result of the displacement of the reference ligand by scrambled peptides.

SUMMARY:

BSUM(10)

"Semisynthetic" . . . have been prepared by (1) limited proteolysis of naturally occurring polypeptides to yield a workable set of fragments, (2) chemical **synthesis** of an additional oligopeptide, and (3) reconstruction of **synthetic** and native partners. The technique is typically used to prepare analogues of naturally occurring polypeptides. Chaiken, CRC Critical Reviews in Biochemistry, 255 (Sept. 1981). Ruggeri, et al., P.N.A.S. (U.S.A.), 83: 5708-12 (Aug. 1986) prepared a series of **synthetic** peptides in lengths up to 16 residues that were modeled on various platelet-binding peptides. The technique used was one of **solid** state **synthesis** by chemical means, but using individual compartmentalized peptide resins to impart the desired variety. See Houghten, et al., P.N.A.S. (U.S.A.),. . . of mixed oligonucleotides. See Goff, et al., DNA, 6: 381-88 (1987). None of these approaches involve a balanced equilibrium between **random** **synthesis** and **random** degradation within a large population of peptides. Instead, a known peptide is cleaved at a known site, a known amino. . . of a particular peptide having a desired binding activity but a then-unknown amino acid sequence will result in net selective **synthesis** of that product without net **synthesis** of large amounts of every possible product.

SUMMARY:

BSUM(11)

The . . . peptide synthesis has previously been directed toward the synthesis of single, known peptide species. Several patents relate to such enzymatic **synthesis** of non-**random** peptides and, to this end, disclose and claim use of protective groups to prevent formation of other peptides. Isowa, U.S.. . .

SUMMARY:

BSUM(16)

Pieczenik, . . . of fractionated DNA into an expression vector and subsequent expression thereof. Pieczenik does not, however, recognize the advantages of combining **random** degradation with **random** **synthesis** within a **chemical** system wherein they are in a balanced equilibrium, and disturbing that equilibrium only to favor synthesis of a peptide species. . .

SUMMARY:

BSUM(20)

A . . . purposes. The method further provides for the generation of analyzable (picomole to millimole) quantities of such peptides without concomitant net **synthesis** of such quantities of extraneous **random** peptides. A full showing of pharmacologic utility of a single peptide isolated from a large, random population of peptides involves. . . of the specific binding peptide, (4) determination of the structure (amino acid sequence) of the specific binding peptide, (5) large-scale **synthesis** of the specific binding peptide, (6) demonstration of biological activity of peptide, and (7) pre-clinical and clinical testing. Satisfactory methods. . . proteases to randomly generate large numbers of peptides of a particular size and of varied, undetermined sequences through balanced peptide **synthesis** and degradation, (2) a molecular trap to drive the **synthetic** reaction toward completion with respect to only the binding peptide, as well as to screen for the particular peptide(s) of interest, and (3) a semi-permeable barrier, covalent or **matrix** immobilization, or similar means of physically separating the scrambling system from the molecular trap. An object of the invention is to provide a means for inexpensive and rapid **synthesis** of very large **random** populations of peptides. Another object of the invention is to provide a method for screening such a population for the. . .

DETDESC:

DETD(25)

In . . . temperature (25.degree. C.) the digestion requires about two hours. The digested material is fractionated by gel-permeation chromatography on a polyacrylamide **matrix** (e.g., Bio-Rad, Bio-GelM). Fractions containing peptides of about 7-12 a.a. are collected, lyophilized and reconstituted at a concentration of about. . . adjusted by either adding more protein, which will increase the average peptide length (i.e. higher concentration of a.a. and increased **synthesis**), or diluting the peptide mixture to decrease the peptide chain length (i.e. increased hydrolysis of existing peptides). The actual peptide. . .

DETDESC:

DETD(84)

In . . . forms of chromatography: gel filtration, reversed phase, isoelectric focusing, ion exchange, normal phase, partition, etc. The effluent from the chromatography **matrix** is analyzed for both 214 nM UV (all peptides) and .sup.3 H (those having undergone **synthesis**) detection. Any increase in chemical heterogeneity, as measured by .sup.3 H incorporation into new peptides derived from the unlabelled starting. . .

DETDESC:

DETD(92)

If . . . radiolabeled before it is placed in the binding compartment, and the level of radioactivity in the sample aliquot measured. If **labeling** the target before it is placed in the binding compartment would alter its binding characteristics, it may be **labeled** after it is withdrawn in the sample aliquot, e.g., with a **labeled** antibody. In any event, any method of measuring the amount of target in the aliquot taken from the soluble fraction. . . compartment, which does not substantially interfere with the purpose of the invention is suitable for use as part of this **monitoring** method.

DETDESC:

DETD(161)

For . . . concentration >10.sup.-13 molar) is therefore 10.sup.-13 moles. However, if the concentration of each peptide is maintained at 10.sup.-13 molar (by **random** peptide **synthesis**), the maximum amount of a single peptide which can be trapped is both a function of the Kd of the. . .

DETDESC:

DETD(202)

Monitoring the **Synthesis** and Binding of Functional Peptides to the Receptor Macromolecule

DETDESC:

DETD(209)

Given . . . limit of detection for free iodinated fragment D. Thus, an increase in free iodinated fragment D would serve as a **monitor** for the **synthesis** of peptides with higher affinity for the GPRP binding site on fragment D.

4. 5,284,935, Feb. 8, 1994, MHC-mediated toxic conjugates useful in ameliorating autoimmunity; Brian R. Clark, et al., 424/185.1, 193.1, 810; 530/395, 403, 806, 807, 868 [IMAGE AVAILABLE]

US PAT NO: 5,284,935 [IMAGE AVAILABLE] L5: 4 of 7

ABSTRACT:
The invention is directed to methods and materials useful in treating autoimmune diseases. The therapeutic agents are of the formula X--MHC--peptide or MHC--peptide--X wherein X represents a functional moiety selected from a toxin and a labeling group; MHC is an effective portion of the MHC glycoprotein, said glycoprotein dissociated from the cell surface on which it normally resides; and "peptide" represents an antigenic peptide sequence associated with an autoantigen;--represents a covalent bond or a linker bound to X and MHC or to X and peptide by covalent bonds; and--represents a covalent bond, a noncovalent association, or a linker covalently bound to or associated with the MHC and peptide. These complexes can be used to target helper T-cells which are specifically immunoreactive with autoantigens.

SUMMARY:

BSUM(28)

Yet another aspect of the invention is a method to **monitor** an autoimmune disease in a vertebrate subject which method comprises administering to a vertebrate in need of such treatment the complex or a pharmaceutical composition in which the complex is the active ingredient, wherein X is a **label**.

DETDESC:

DETD(39)

The Dupont apparatus and technique for raoid **multiple** peptide **synthesis** (RAMPS) is used to **synthesize** the members of a set of overlapping (10 residue overlap), 20-residue peptides from the alpha subunit of Torpedo californicus AChR.. . . this peptide is known and is shown in FIG. 6. One or more radioactive amino acids is incorporated into each **synthetic** peptide. The pentafluorphenyl active esters of side chain-protected, FMOC amino acids are used to **synthesize** the peptides, applying standard stepwise **solid** phase peptide **synthetic** methods, followed by standard side chain deprotection and simultaneous release of the peptide amide from the **solid** **support**.

DETDESC:

DETD(47)

The identified peptides are then prepared by conventional **solid** phase **synthesis** and the subset which contain epitopes for the disease-inducing helper T-cell clones i determined by incubation of the candidate peptides. . .

DETDESC:

DETD(75)

A . . . individuals T cells are examined in vitro, to determine the autopeptide(s) recognized by autoreactive T cells; this is accomplished utilizing **labeled** complexes of the invention, described supra., which are of the formula X--MH--peptide, wherein X is a **label** moiety. After it is determined which complexes target the T-cells, the individual is treated with complexes of the invention which. . . (wherein X is a moiety capable of killing the T-cell), respectively. Therapy (as determined by the autoreactive T-cells remaining) is **monitored** with T-cell binding studies using the **labeled** complexes of the invention, described supra.

DETDESC:

DETD(121)

An iodinated **synthetic** peptide representing amino acids 1-13 of bovine myelin-basic protein (BNBP) is **synthesized** using standard **solid** phase **synthesis** for FMOC protected amino acids. The resulting peptide has the sequence Ac-Ala-Ser-Ala-Gln-Lys-Arg-Pro-Ser-Gln-Arg-Ser-Lys-(I-131)Tyr-NH.sub.2. MHCII is purified from spleen cells of PL/J. . . (supra) incorporated herein by reference. The purified MHCII in detergent or as a lipid bilayer (suora) is incubated with the **synthesized** peptide until the radiolabel uptake into the high molecular weight fraction is optimized. The excess radiolabeled peptide is then removed. . .

DETDESC:

DETD(138)

The . . . in a proliferation assay. Uptake of 3-(4,5-dimethyl-thiazol-2-7)-2,5 diphenyltetrazolium bromide (MTT) was used as an indication of cell proliferation. Although DNA **synthesis**, usually **monitored** by .sup.3 H-thymidine uptake, and the activity of mitochondria, measured by MTT uptake, are different cellular functions, it has been. . .

5. 5,260,422, Nov. 9, 1993, MHC conjugates useful in ameliorating autoimmunity; Brian R. Clark, et al., 424/185.1, 193.1. 810; 530/402, 403, 868 [IMAGE AVAILABLE]

US PAT NO: 5,260,422 [IMAGE AVAILABLE] L5: 5 of 7

ABSTRACT:
The present invention is directed to complexes consisting essentially of an isolated MHC component and an autoantigenic peptide associated with the antigen binding site of the MHC component. These complexes are useful in treating autoimmune disease.

DETDESC:

DETD(38)

The Dupont apparatus and technique for rapid **multiple** peptide **synthesis** (RAMPS) is used to **synthesize** the members of a set of overlapping (10 residue overlap), 20-residue peptides from the alpha subunit of Torpedo californicus AChR.. . . this peptide is known and is shown in FIG. 6. One or more radioactive amino acids is incorporated into each **synthetic** peptide. The pentafluorphenyl active esters of side chain-protected, FMOC amino acids are used to **synthesize** the peptides, applying standard stepwise **solid** phase peptide **synthetic** methods, followed by standard side chain deprotection and simultaneous release of the peptide amide from the **solid** **support**.

DETDESC:

DETD(46)

The identified peptides are then prepared by conventional **solid** phase **synthesis** and the subset which contain epitopes for the disease-inducing helper T cell clones is determined by incubation of the candidate. . .

DETDESC:

DETD(69)

A . . . individuals T cells are examined in vitro, to determine the autopeptide(s) recognized by autoreactive T cells; this is accomplished utilizing **labeled** complexes of the invention, described supra, which are of the formula X.sub.--.sup.1 MHC.sub.--.sup.2 peptide, wherein X is a **label** moiety. After it is determined which complexes target the T cells, the individual is treated with complexes of the invention. . . is a moiety capable of killing the T cell), respectively. Therapy (as determined by the autoreactive T cells remaining) is **monitored** with T cell binding studies using the **labeled** complexes of the invention, described supra.

DETDESC:

DETD(127)

The . . . in a proliferation assay. Uptake of 3-(4,5-dimethyl-thiazol-2-7)-2,5 diphenyltetrazolium bromide (MTT) was used as an indication of cell proliferation. Although DNA **synthesis**, usually **monitored** by .sup.3 H-thymidine uptake, and the activity of mitochondria, measured by MTT uptake, are different cellular functions, it has been. . .

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=> s l2 and (label#### (P)monitor###### (P) synthe####)

113286 LABEL####
256602 MONITOR######
322138 SYNTHE####
188 LABEL#### (P)MONITOR###### (P) SYNTHE####
L6 22 L2 AND (LABEL#### (P)MONITOR###### (P) SYNTHE####)

=> s l6 not l5

L7 20 L6 NOT L5

=> d 1-20 cit ab hit

1. 5,723,591, Mar. 3, 1998, Self-quenching fluorescence probe; Kenneth J. Livak, et al., 536/22.1, 23.1, 24.3, 25.3, 25.32 [IMAGE AVAILABLE]

US PAT NO: 5,723,591 [IMAGE AVAILABLE] L7: 1 of 20

ABSTRACT:
An oligonucleotide probe is provided which includes a fluorescent reporter molecule and a quencher molecule capable of quenching the fluorescence of the reporter molecule. The oligonucleotide probe is constructed such that the probe exists in at least one single-stranded conformation when unhybridized where the quencher molecule is near enough to the reporter molecule to quench the fluorescence of the reporter molecule. The oligonucleotide probe also exists in at least one conformation when hybridized to a target polynucleotide where the quencher molecule is not positioned close enough to the reporter molecule to quench the fluorescence of the reporter molecule. By adopting these hybridized and unhybridized conformations, the reporter molecule and quencher molecule on the probe exhibit different fluorescence signal intensities when the probe is hybridized and unhybridized. As a result, it is possible to determine whether the probe is hybridized or unhybridized based on a change in the fluorescence intensity of the reporter molecule, the quencher molecule, or a combination thereof. In addition, because the probe can be designed such that the quencher molecule quenches the reporter molecule when the probe is not hybridized, the probe can be designed such that the reporter molecule exhibits limited fluorescence until the probe is either hybridized or digested.

SUMMARY:

BSUM(27)

A wide variety of linkers are known in the art which may be used to attach the oligonucleotide probe to the **solid** **support**. The linker most preferably includes a functionalized polyethylene glycol because it does not significantly interfere with the hybridization of probe to the target oligonucleotide, is commercially available, soluble in both organic and aqueous media, easy to functionalize, and completely stable under oligonucleotide **synthesis** and post-**synthesis** conditions.

DETDESC:

DETD(10)

Examples of preferred types of **solid** supports for immobilization of the oligonucleotide probe include controlled pore glass, glass plates, polystyrene, avidin coated polystyrene beads, cellulose, nylon, acrylamide gel and activated dextran. CPG, glass plates and high cross-linked polystyrene. These **solid** supports are preferred for hybridization and diagnostic studies because of their chemical stability, ease of functionalization and well defined surface area. **Solid** supports such as controlled pore glass (CPG, 500 .ANG., 1000 .ANG.) and

non-swelling high cross-linked polystyrene (1000 .ANG.) are particularly preferred in view of their compatibility with oligonucleotide **synthesis**.

DETDESC:

DETD(12)

The length and chemical stability of linker between **solid** **support** and the first 3' unit of oligonucleotides play an important role in efficient **synthesis** and hybridization of **support** bound oligonucleotides. The linker arm should be sufficiently long so that a high yield (>97%) can be achieved during automated **synthesis**. The required length of the linker will depend on the particular **solid** **support** used. For example, a six atom linker is generally sufficient to achieve a >97% yield during automated **synthesis** of oligonucleotides when high cross-linked polystyrene is used as the **solid** **support**. The linker arm is preferably at least 20 atoms long in order to attain a high yield (>97%) during automated **synthesis** when CPG is used as the **solid** **support**.

DETDESC:

DETD(13)

Hybridization of a probe immobilized to a **solid** **support** generally requires that the probe be separated from the **solid** **support** by at least 30 atoms, more preferably at least 50 atoms. In order to achieve this separation, the linker generally includes a spacer positioned between the linker and the 3' nucleoside. For oligonucleotide **synthesis**, the linker arm is usually attached to the 3'-OH of the 3' nucleoside by an ester linkage which can be cleaved with basic reagents to free the oligonucleotide from the **solid** **support**.

<-----User Break----->

DETD(14)

A wide variety of linkers are known in the art which may be used to attach the oligonucleotide probe to the **solid** **support**. The linker may be formed of any compound which does not significantly interfere with the hybridization of the target sequence to the probe attached to the **solid** **support**. The linker may be formed of a homopolymeric oligonucleotide which can be readily added on to the linker by automated **synthesis**. Alternatively, polymers such as functionalized polyethylene glycol can be used as the linker. Such polymers are preferred over homopolymeric oligonucleotides because they do not significantly interfere with the hybridization of probe to the target oligonucleotide. Polyethylene glycol is particularly preferred because it is commercially available, soluble in both organic and aqueous media, easy to functionalize, and completely stable under oligonucleotide **synthesis** and post-**synthesis** conditions.

DETDESC:

DETD(20)

Use of an oligonucleotide probe according to the present invention for **monitoring** nucleic acid amplification provides several advantages over the use of prior art reporter-quencher pair probes. For example, prior art probes required that the reporter and quencher molecules be positioned on the probe such that the quencher molecule remained within a minimum quenching distance of the reporter molecule. However, by realizing that the probe need only be designed such that the probe be able to adopt a conformation where the quencher molecule is within a minimum quenching distance of the reporter **molecule**, a far wider **array** of probes are enabled. For example, dually **labelled** probes having the reporter and quencher molecules at the 5' and 3' ends can be designed. Such probes are far easier to **synthesize** than probes where the reporter molecule or the quencher molecule is attached to an internal nucleotide. Positioning of the reporter and quencher molecules on terminal nucleotides also enhances the hybridization efficiency of the probes.

DETDESC:

DETD(33)

Rhodamine and fluorescein dyes are also conveniently attached to the 5' hydroxyl of an oligonucleotide at the conclusion of **solid** phase **synthesis** by way of dyes derivatized with a phosphoramidite moiety, e.g., Woo et al., U.S. Pat. No. 5,231,191; and Hobbs, Jr., U.S. Pat. No. 4,997,928.

DETDESC:

DETD(42)

2. **Synthesis** of Oligonucleotide Probes Attached to a **Solid** **Support**

DETDESC:

DETD(44)

Table 2 illustrates a reaction scheme for the **synthesis** of a spacer, compound 5, which is used to derivatize CPG and polystyrene supports. As shown in Table 2, N-Fmoc-.epsilon.-aminocaproic acid was reacted with DL-homoserine in presence of HOBT/HBTU/DIPEA (Knorr, et al., Tetrahedron Lett. 1989, 30, 1927) in DMF to give compound 2 in 65% yield. Compound 2 was reacted with dimethoxytrityl chloride in presence of DMAP in pyridine to give compound 3 in 72% yield after chromatography. Treatment of compound 3 with a large excess of PEG-diamine (Buckmann, et al., Biotech. Appl. Biochem. 1987, 9, 258) in presence of HOBT/HBTU/DIPEA in DMF afforded amine 4 in 60% yield. The amine 4 was then converted to succinate 5 by treating amine 4 with succinic anhydride/Et.sub.3 N/DMAP in CH.sub.2 Cl.sub.2 in 90% yield. The succinate 5 was then attached to polystyrene and CPG **support** as illustrated in Tables 3 and 4 respectively without further purification.

DETDESC:

DETD(46)

Double labeled Taqman probe was **synthesized** using both TAMRA labeled supports 7 and 9, FastPhoramidites (User Bulletin Number 85, Perkin Elmer Corporation 1994) and FAM phosphoramidite (User Bulletin Number 78, Perkin Elmer Corporation 1994) in 40 nanomol scale. The **support** bound oligonucleotides were deprotected by treating with MeOH:t-BuNH.sub.2 :H.sub.2 O (1:1:2) at 65.degree. C. for 3 hours (Woo, et al., U.S. Pat. No. 5,231,191). Liquid was removed and the **support** containing probes were washed with H.sub.2 O:MeOH (3:1) and MeOH. The **support** was then dried under vacuum and used in a hybridization assay.

DETDESC:

DETD(54)

Synthesis of FAM and TAMRA Doubled Labeled Probes: Doubled dye labeled probes were **synthesized** by using TAMRA labelled supports 7 and 9, DNA FastPhosphoramidite and FAM amidite in 40 nmol scale. After completion of **synthesis**, supports containing probes were transferred to 4 mL glass vials and treated with a mixture of MeOH:t-BuNH.sub.2 :H.sub.2 O (1:1:2) at 65.degree. C. for 3 hours. Liquid was removed by a syringe and the **support** was washed with H.sub.2 O:MeOH (3:1) and MeOH. The **support** was dried under vacuum and used in the hybridization assay.

2. 5,700,906, Dec. 23, 1997, Immunogenic peptide antigen corresponding to plasmodium vivax circumsporozoite protein; David E. Arnot, et al., 530/324, 300, 326, 350 [IMAGE AVAILABLE]

US PAT NO: 5,700,906 [IMAGE AVAILABLE] L7: 2 of 20

ABSTRACT:

A synthetic peptide comprising an amino acid sequence including the sequence Asp-Arg-Ala-X-Gly-Gln-Pro-Ala-Gly wherein X is selected from the group consisting of Asp and Ala, said peptide being capable of eliciting formation of antibodies recognizing the circumsporozoite protein of Plasmodium vivax sporozoites.

SUMMARY:

BSUM(20)

CS proteins have been identified, analyzed and characterized for some plasmodium species including P. falciparum. Synthetic peptides (produced by **chemical** **synthesis** or biological methods) consisting of **multiples** or analogs of the repeating amino acid sequences have been shown to be immunogenic and are useful in the development of a malaria vaccine.

DETDESC:

DETD(11)

To confirm the deduced amino acid sequence and immunochemical reactivity of the above-described sequence, a dodecapeptide of the same amino acid sequence and a dimer thereof were **synthesized** using an automated **solid** phase peptide **synthesis** system.

DETDESC:

DETD(77)

The peptide was **synthesized** using the stepwise **solid**-phase method of R. B. Merrifield, J. Am. Chem. Soc. 85:2149 (1963) on a multidetachable benzhydrylamine resin (p-acyloxy benzhydrylamine copolystyrene 1% divinyl benzene resin) as described by Tam, J. P. et al., Tetrahedron Lett., 2851 (1981). Boc-Ala-p-acyloxybenzhydrylamine resin (0.4 mmol per gram substitution of resin) was placed into the reaction vessel of a Beckman 990M **synthesizer** and a double coupling protocol via dicyclohexylcarbodiimide was used to give a coupling efficiency more than 99.85% completion per step. The benzyl-based side chain protecting groups and tert-butoxycarbonyl (Boc) for the N-alpha terminus were used. The sequence Asp-Gly is prone to a cyclization reaction to form aspartimide during the **synthesis** and acid deprotection of the protected peptide-resin. To prevent base catalyzed aspartimide formation during the **synthesis** and the strong-acid catalyzed deprotection step, a new protection group, Asp (OcHex, aspartyl-Beta cyclohexyl ester) was used as described by Tam, J. P. et al., Tetrahedron Lett. 4033, (1979), incorporated herein by reference. The unpurified peptide, when examined by high pressure liquid chromatography gave a single symmetrical peak accounting for more than 83% of all peptide content. The crude peptide was purified by preparative low-pressure liquid chromatography (100-120 psi) on a reversed-phase C-18 column (2.5.times.30 cm) using aqueous CF.sub.3 CO.sub.2 H (0.05%) and acetonitrile gradient. The purified material gave a single symmetrical peak on analytical high pressure liquid chromatography and an amino acid analysis gave the correct theoretical values of amino acids. The overall yield based on the first alanine attached to the resin was 72%.

DETDESC:

DETD(116)

To confirm that the foregoing amino acid sequence contains the immunoreactive site, a dodecapeptide (with the same order of amino acids as shown above) and a dimer of the dodecapeptide have been **synthesized**, using **solid** phase resin **synthesis** (Marglin, H. and Merrifield, R. B., Ann. Rev. Biochem. 39:841-866 (1970). Sequence analysis performed by automated Edman degradation confirmed that the peptide had been correctly **synthesized**. The final proof that this is the correct epitope has been obtained. Rabbits were immunized with the dodecapeptide coupled to a carrier (bovine gamma globulin in complete Freunds adjuvant). Two weeks after the injection, the rabbits were bled and their serum assayed for the presence of antibodies against the dodecapeptide and against extracts of sporozoites. The results showed that the animals produced high titers (greater than 1:1000) of antibodies to the native CS protein present in the parasite extracts.

DETDESC:

DETD(175)

TABLE V

Results of two-site immunoradiometric assay performed with monoclonal antibody 2G3 and **synthetic** peptides

Concentration of 12-Mer or 24-Mer incubated with the **solid**-phase antibody 2G3 (ug/ml)	Amount of radiolabeled 2G3 (cpm) bound in wells incubated with 12-MER	24-MER
500.	157	5517
50.	103	2056
5.	40	402
.5	0	93
.05	–	35

DETDESC:

DETD(190)

4 mg of the carrier protein in 0.25 ml of 0.05M PO.sub.4 buffer, pH 7.2, is reacted dropwise with 0.7 mg MBS dissolved in dimethyl-formamide, and stirred for 30 min. at room temperature. The product, that is, MB-carrier, is separated from the unreacted chemicals by passage in a Sephadex G-25 column equilibrated in 0.05M PO.sub.4 buffer, pH 6.0. The MB-carrier is then reacted with 5 mg of the 24-MER containing cysteine, dissolved in PBS (pH 7.4). The mixture is stirred for 3 hours at room temperature. Coupling efficiency is **monitored** with radioactive peptide; that is, a trace amount of .sup.125 I-**labeled** 24-MER is mixed with cold peptide during the **synthesis**. Dialysis of the conjugate permits evaluation of the proportion of incorporated **label**. The number of 24-MER groups per 100,000 M.W. carrier was estimated to be about 10-14.

DETDESC:

DETD(196)

4 mg of the carrier protein in 0.25 ml of 0.05M PO.sub.4 buffer, pH 7.2., is reacted dropwise with 0.7 mg MBS dissolved in dimethyl-formamide, and stirred for 30 min. at room temperature. The product, that is, MB-carrier, is separated from the unreacted chemicals by passage in a Sephadex G-25 column equilibrated in 0.05M PO.sub.4 buffer, pH 6.0. The MB-carrier is then reacted with 5 mg of each peptide containing cysteine, dissolved in PBS (pH 7.4). The mixture is stirred for 3 hours at room temperature. Coupling efficiency is **monitored** with radioactive peptide; that is, a trace amount of .sup.125 I-**labelled** is mixed with cold peptide during the **synthesis**. Dialysis of the conjugate permits evaluation of the proportion of incorporated **label**. The number of **synthetic** peptides per 100,000 H. W. Carrier is estimated to be about 10-14.

3. 5,691,142, Nov. 25, 1997, Detection of target nucleic acid molecules using synthesis-deficient thermostable DNA polymerase; James E. Dahlberg, et al., 435/6, 91.2, 183; 436/94; 536/24.3, 24.33; 935/78 [IMAGE AVAILABLE]

US PAT NO: 5,691,142 [IMAGE AVAILABLE] L7: 3 of 20

ABSTRACT:

A means for cleaving a nucleic acid cleavage structure in a site-specific manner is disclosed. A cleaving enzyme having 5' nuclease activity without interfering nucleic acid synthetic ability is employed as the basis of a novel method of detection of specific nucleic acid sequences. In this novel detection method, the signal indicative of the presence of the target nucleic acid sequence is amplified through cleavage of detection molecule(s) which comprise hairpin structures.

SUMMARY:

BSUM(12)

The immobilization of target nucleic acids to solid surfaces such as nylon or nitrocellulose is a common practice in molecular biology. Immobilization formats eliminate the reassociation problem that can occur between complementary strands of target molecules, but not the problems associated with secondary structure effects. However, these mixed phase formats (i.e., Southern hybridization or dot blot hybridization) require time consuming fixation procedures. The hybridization reaction itself is kinetically much slower than a solution phase hybridization reaction. Together, the fixation and hybridization procedures require a minimum of several hours to several days to perform. Additionally, the standard immobilization procedures are often inefficient and result in the attachment of many of the target **molecules** to **multiple** portions on the solid surface, rendering them incapable of subsequent hybridization to probe molecules. Overall, these combined effects result in just a few percent of the initial target molecules being bound by probes in a hybridization reaction.

DETDESC:

DETD(78)

The following is an example of the dual capture assay to detect an antigen(s): A sample to be analyzed for a given antigen(s) is provided. This sample may comprise a mixture of cells; for example, cells infected with viruses display virally-encoded antigens on their surface. If the antigen(s) to be detected are present in solution, they are first attached to a **solid** **support** such as the wall of a microtiter dish or to a bead using conventional methodologies. The sample is then mixed with 1) the **synthetic** domain of a thermostable DNA polymerase conjugated to an antibody which recognizes either a first antigen or a first epitope on an antigen, and 2) the 5' nuclease domain of a thermostable DNA polymerase conjugated to a second antibody which recognizes either a second, distinct antigen or a second epitope on the same antigen as recognized by the antibody conjugated to the **synthetic** domain. Following an appropriate period to allow the interaction of the antibodies with their cognate antigens (conditions will vary depending upon the antibodies used; appropriate conditions are well known in the art), the sample is then washed to remove unbound antibody-enzyme domain complexes. dATP, dTTP and a small amount of poly d(A-T) is then added to the washed sample and the sample is incubated at elevated temperatures (generally in the range of 60.degree.-80.degree. C. and more preferably, 70.degree.-75.degree. C.) to permit the thermostable **synthetic** and 5' nuclease domains to function. If the sample contains the antigen(s) recognized by both separately conjugated domains of the polymerase, then an exponential increase in poly d(A-T) production occurs. If only the antibody conjugated to the **synthetic** domain of the polymerase is present in the sample such that no 5' nuclease domain is present in the washed sample, then only an arithmetic increase in poly d(A-T) is possible. The reaction conditions may be controlled in such a way so that an arithmetic increase in poly d(A-T) is below the threshold of detection. This may be accomplished by controlling the length of time the reaction is allowed to proceed or by adding so little poly d(A-T) to act as template that in the absence of nuclease activity to generate new poly d(A-T) primers very little poly d(A-T) is **synthesized**.

DETDESC:

DETD(82)

1) use of a radioactive **label** on either the dATP or dTTP supplied for the **synthesis** of the poly d(A-T), followed by size separation of the reaction products and autoradiography; 2) use of a fluorescent probe on the dATP and a biotinylated probe on the dTTP supplied for the **synthesis** of the poly d(A-T), followed by passage of the reaction products over an avidin bead, such as magnetic beads conjugated to avidin; the presence of the florescent probe on the avidin-containing bead indicates that poly d(A-T) has been formed as the fluorescent probe will stick to the avidin bead only if the fluorescenated dATP is incorporated into a covalent linkage with the biotinylated dTTP; and 3) changes fluorescence polarization indicating an increase in size. Other means of detecting the presence of poly d(A-T) include the use of intercalating fluorescence indicators to **monitor** the increase in duplex DNA formation.

DETDESC:

DETD(108)

To determine whether other 5' nucleases in other DNAPs would be suitable for the present invention, an **array** of enzymes, several of which were reported in the literature to be free of apparent 5' nuclease activity, were examined. The ability of these other enzymes to cleave nucleic acids in a structure-specific manner was tested using the hairpin substrate shown in FIG. 6 under conditions reported to be optimal for **synthesis** by each enzyme.

4. 5,688,486, Nov. 18, 1997, Use of fullerenes in diagnostic and/or therapeutic agents; Alan D. Watson, et al., 424/1.65, 9.36, 9.42 [IMAGE AVAILABLE]

US PAT NO: 5,688,486 [IMAGE AVAILABLE] L7: 4 of 20

ABSTRACT:

Compounds including tight molecular meshes, preferably curved in one or two directions, such as fullerenes and met-cars, can be used as carriers for diagnostic or therapeutic agents, especially diagnostic contrast agents.

SUMMARY:

BSUM(50)

Further opportunities for modification of molecular weights and loading capacities for diagnostic and therapeutic entities may be achieved by the use of bifunctional linkers, e.g. dihaloalkanes, to produce dimers, trimers or higher oligomers and in the limit polymers of the fullerenes. The oligomers can be derivatised as discussed above to provide chelating groups capable of complexing diagnostic metal ions or to introduce solubilising or other surface bound groups. Similarly a plurality of fullerenes, optionally derivatised, may be conjugated to a backbone, e.g. polymer, molecule (for example a polylysine or a starburst dendrimer) and if desired such a **multiply** fullerene loaded **molecule** can then be conjugated to a targetting molecule, e.g. a biomolecule as discussed above. Examples of backbone structures are discussed in WO-A-90/12050.

DETDESC:

DETD(44)

Gd@C.sub.60 is **synthesized** as described previously (see Example 3). The Gd@C.sub.60 cluster is made water soluble using the method described by Chiang et al., J Am Chem Soc 114:10154-10157 (1992). In a 50 mL round bottom flask is placed 0.176 g (0.2 mmol) of Gd@C.sub.60, nitronium tetrafluoroborate (8.0 mL, 4.0 mmol, 0.5M in sulfolane), benzoic acid (0.488 g, 4 mmol), and dichloromethane (20 mL). The reaction mixture is stirred under a nitrogen atmosphere for 2 days, after which the dichloromethane is removed by evaporation to afford a thick slurry. The slurry is slowly added to 50 mL of ice water, yielding an oil that solidifies on stirring. The **solid** is filtered and washed with hexane. After drying in air, 50 mg of the **solid** is weighed into a 25 mL flask; 10 mL of water and 150 mg of sodium hydroxide are added. The resulting mixture is heated for 8 hr with stirring. The resulting clear solution is concentrated to a volume of 5 mL and added to methanol, whereupon a precipitate of polyhydroxylated Gd@C.sub.60 is obtained. The FAB mass spectrum shows peaks at m/z 878 (Gd@C.sub.60), 894, 911, 927, 943, 961, and higher.

DETDESC:

DETD(72)

Fluorinated fullerenes are prepared by treating brominated fullerenes with appropriate reagents. Thus C.sub.60 Br.sub.24, prepared as described by Tebbe et al., Science, 256:822 (1992) is reacted with a suspension of potassium fluoride in sulfolane as described in J Chem Soc 6264 (1965) to afford a mixture of compounds having formulae C.sub.60 Br.sub.24-x F.sub.x (where x=1, 2, 3, . . .) as indicated by the mass spectrum of the resulting product mixture (m/e clusters being observed at 2638 (C.sub.60 Br.sub.24 parent), 2577 (C.sub.60 Br.sub.23 F), 2516 (C.sub.60 Br.sub.22 F.sub.2), etc.). Use of potassium fluoride **labelled** with the fluorine-18 isotope permits the **synthesis** of fluorine-18labelled brominated C.sub.60 molecules Usable for PET imaging; use of commercially available potassium fluoride (consisting exclusively of fluorine isotope 19) affords materials that can be used for NMR imaging by **monitoring** the resonance of the .sup.19 F nucleus.

5. 5,674,977, Oct. 7, 1997, Branched synthetic peptide conjugate; Jean Gariepy, 530/324, 327, 328, 329, 330, 332, 345; 930/290 [IMAGE AVAILABLE]

US PAT NO: 5,674,977 [IMAGE AVAILABLE] L7: 5 of 20

ABSTRACT:
The invention is a branched synthetic peptide conjugate which can be designed to bind to a target cell surface receptor, to penetrate into target cells, and to deliver a diagnostic probe or cytotoxic functionality to a desired site of action. The invention provides a relatively small molecule of flexible design having a branched structure for systematically incorporating a desired number of cytotoxic functions, peptide-based localization signals or diagnostic probes. The invention addresses problems associated with protein-based therapeutic or diagnostic agents.

SUMMARY:

BSUM(3)

The understanding of mechanisms leading to the endocytosis, vesicular transport and compartmentalization of proteins inside cells has recently been broadened by the discovery of domains in proteins coding for their transport, retention or retrieval into cellular compartments. Cellular events such as retrograde transport and transcytosis have been characterized by the study of bacterial and plant toxins in conjunction with the use of agents such as brefeldin A and cerulenin. Based on this emerging spectrum of molecular and cellular information, an opportunity now exists for designing de novo peptide-based agents that can target selected compartments inside cells. Few systematic approaches presently exist that will allow the design of molecules able to act as intracellular targeting agents. The screening of **chemical** **libraries** using functional or receptor binding assays still represents a common, but random, approach for identifying useful low molecular weight compounds. However, molecular candidates able to perform only one cellular task will most likely prove inadequate in carrying out a series of specific cellular functions. Another viable but relatively long term strategy combines the use of mutagenesis and high resolution structural studies directed at finding useful protein variants based on existing protein designs. For example, the bacterial toxin pseudomonas aeruginosa exotoxin A is a protein composed of three defined functional domains coding for cellular uptake, membrane translocation and cytotoxicity. Its study has led to the engineering of hybrid proteins that retain the toxin's cellular functions but have altered receptor-binding properties (Chaudhary et al., 1987; Lorberboum-Galski et al., 1987; Chaudhary et al., 1988; Siegall et al. 1988; Brinkmann et al., 1992; Kreitman et al., 1993). Unfortunately, the dimensions and structural complexity of such constructs establish important constraints exemplified by their possible immunogenicity, their reduced ability to penetrate **solid** tumours and tissues with altered vasculature, and the lack of flexibility in redesigning simpler constructs. An alternate, but yet untested strategy would be to adopt the minimalist view that domains of proteins or simple polypeptides have already been identified which can code for all necessary transport tasks needed to build prototype intracellular vehicles. A starting requirement is to identify a series of transport tasks and to assemble the required signals onto a flexible scaffold that allows the proper presentation of multiple functional domains. Recent advances in **solid**-phase peptide **synthesis**, particularly in the areas of branched peptide technology (Tam, 1988, 1989), and orthogonal **synthesis** strategies (Field and Noble, 1990), have dramatically broadened the flexibility and ease of creating novel multidomain-containing peptides.

DRAWING DESC:

DRWD(10)

FIG. 6A shows the effects of ATP **synthesis** inhibitors on octopeptide 4Rh uptake by CHO cells. Cells were incubated at 37.degree. C. in the presence (.diamond-**solid**.) or absence (.quadrature.) of NaN.sub.3 (5 mM) and 2-deoxyglucose (10 mM).

DETDESC:

DETD(4)

The branched peptides of the invention are readily **synthesized** using existing or modified procedures of classical **solid** phase peptide **synthesis**. In addition to being rapid and simple, **solid** phase peptide **synthesis** permits the removal of unreacted reagents and by-products at each step of the **synthesis**. Also, each **synthetic** step may be repeated until satisfactory yields are obtained.

DETDESC:

DETD(28)

Octopeptide 5 is a branched peptide containing the DNA intercalator acridine (Acr) that is able to cross the cytoplasmic membrane of cells and accumulate in the cell nucleus. Octopeptide 5 is composed of eight N-terminal branches and one C-terminal arm. The branches are identical and composed of a linear arrangement of three domains; the DNA intercalator agent acridine (Acr; D.sup.3), a 12-amino acid sequence of the SV40 large T antigen that is responsible for the nuclear translocation of this protein (NLS; nuclear localization signal; D.sup.2) and a 5-residue linear lysine repeat (CTS, cytoplasm translocation signal; D.sup.1). These branches are linked to a branched polymer (BP) via a junctional segment (J.sup.1) composed of two glycine residues. BP is created after three successive couplings of L-lysine during **solid**-phase peptide **synthesis**. The efficiency of the peptide to cross the cytoplasmic membrane is dependent on the level of cationic charges present on the peptide. The presence of 8 branches carrying the CTS repeat dramatically augments the rate of entry of the branched peptide into cells when compared to an individual branch (i.e., peptide 1). The C-terminal arm (J.sup.4) is composed of three residues used in the analytical evaluation of the construct; tyrosine can be radiolabeled and its absorbance measured at 280 nm, glycine and .beta.-alanine are amino acid standards to monitor the concentration and amino acid composition of the construct.

DETDESC:

DETD(29)

Octopeptide 5 was prepared by **solid** phase peptide **synthesis** on an automated Applied Biosystems model 430A Peptide **Synthesizer** using t-Boc protected amino acids and PAM (phenylacetamidomethyl) resin supports. A similar octopeptide can be generated using Fmoc amino acids and acid sensitive resin supports. Unless indicated, all coupling steps were carried out for 1 to 2 hours at room temperature using symmetric anhydride derivatives of protected amino acids dissolved in dimethylformamide. Each coupling step was then repeated in 10% (v/v) hexafluoroisopropanol in dichloromethane. In the case of arginine and glutamine derivatives, HOBt esters were prepared in dimethylformamide and the coupling step carried out in the same solvent. All **synthesis** protocols employed were those established by the manufacturer (Applied Biosystems, Foster City, Calif.). Each coupling step was monitored by the quantitative determination of free amino groups present on the resin (quantitative ninhydrin test). Typically, the efficiency of each coupling step was greater than 99%. The first residue coupled to the PAM resin was .beta.-alanine (.beta.-aminopropionic acid) and the substitution on the resin **support** was 0.1 mmole/gram of resin. The initial low substitution value on the resin insures that crowding on the resin with peptide chains will not occur as a result of three branching steps (i.e., maximal substitution of 2.sup.3 .times.0.1 mmole=0.8 mmole/gram of resin). The .beta.-alanine serves as an internal standard. The second and third residues were glycine and tyrosine respectively and constitute with .beta.-alanine, an analytical spacer arm that permits one to assess the quality of the **synthesis** (post-**synthesis** amino acid analysis) and the concentration of the polymer (tyrosine side chain absorbs strongly at 280 nm and can be readily radiolabeled with iodine isotopes) in solution. The fourth residue was N.alpha.(Boc),N.epsilon.(Boc)-lysine, an amino acid having its amino groups at the C.alpha. and C.epsilon. positions protected with acid labile Boc protecting groups. After deprotecting these sites with TFA, branching is initiated by coupling two equivalents of N.alpha.(Boc),N.epsilon.(Boc)-lysine to the two available amino positions. After another round of acid deprotection, the branching step was repeated with this time four amino sites available for coupling. N.alpha.(Boc),N.epsilon.(Boc)-lysine was coupled again. The Boc groups on the completed BP domain were deprotected with TFA to expose 8 free amino groups thus allowing the construction of 8 N-terminal arms (n=8). Two glycine(Boc) residues were successively coupled again to act as a spacer (J.sup.1) before introducing five consecutive N.alpha.(Boc),N.epsilon.(2-Cl-Z)-L-lysine groups. These 5 lysine residues (SEQ ID NO:2) constitute a domain (D.sup.1) called the cytoplasm translocation signal or CTS. The presence of 8 of these CTS domains in octopeptide 5 results in a final molecule with a high level of cationic charges and a potential for this octopeptide to be rapidly internalized by cells. The following 12-amino acid sequence (domain D.sup.2) was then introduced; Thr-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-Glu-Asp-Pro (SEQ ID NO:1). This sequence represents a nuclear localization signal or NLS (residues 124 to 135) for the protein known as the SV40 large T antigen. The NLS antigen was added to each branch in a stepwise fashion with each amino acid in this sequence being coupled one at the time to the growing peptide chain starting with proline-135 (the **synthesis** is proceeding from the C-terminus to the N-terminus). As the name suggests, the presence of the NLS domain (D.sup.2) allows octopeptide 5 to be selectively transported to the

nucleus of cells. Although 8 NLS domains are present in this octopeptide construct, a single NLS domain should be sufficient to target this molecule to the nucleus of cells. Junctional segments J.sup.2 and J.sup.3 are absent in octopeptide 5. The final domain introduced in octopeptide 5 is the acridine moiety (D.sup.3). The Boc group of threonine-124 was removed in TFA to expose its N.alpha. amino group. The peptide-resin was resuspended in 9-phenoxyacridine dissolved in hot dried/recrystallized phenol and the resulting slurry was mixed with a stirring bar at 80.degree. C. for 16 hours. This coupling step typically exceeded 98% coupling efficiency as determined by ninhydrin analysis of residual free amino groups on the resin. Finally, octopeptide 5 was detached from the **support** by exposing the peptide-resin to anisole:dimethylsulfide:anhydrous hydrogen fluoride (1:1:10) for 90 minutes at -5.degree. C. The resin was extracted with several ether washes to remove anisole, dimethylsulfide and cleaved protecting groups. The branched peptide was then recovered by extracting the resin with 50% (v/v) acetic acid and lyophilized.

DETDESC:

DETD(30)

The skilled person will appreciate that the number of N-terminal amino groups can be increased readily to 16 or more, with the upper limit depending on the ability to resolve problems of low coupling rates associated with the crowding of the resin **support** and the accessibility of free amino groups. The rapidity of the **synthesis** strategy and its potential for automation represent major advantages in developing the technology. With an expected molecular weight of 17,878 daltons, octopeptide 5 was desalted on a Sephadex G-25 column equilibrated in distilled water to remove low molecular weight impurities resulting from the cleavage step. The recovered octopeptide 5 has 8 (.+-.1) acridine groups and the correct amino acid composition.

DETDESC:

DETD(38)

Attempts were originally made to use acridine-containing peptides in flow cytometry experiments. However, the available laser excitation lines did not produce strong emission signals for this chromophore during preliminary analyses. Since octopeptide 4Rh contains a single rhodamine probe as well as both CTS and NLS domains, it represented an analog more suited to the analysis of cellular events by flow cytometry. As discussed above, it was observed that octopeptide 5Rh only associated with the cytoplasmic membrane at 4.degree. C. (FIGS. 3A, 3B). Similarly, exposing CHO cells at 4.degree. C. to all octopeptides resulted in the accumulation of fluorescence signal at the perimeter of the cytoplasmic membrane (results not shown). Using flow cytometry, it was established that in less than one hour, all CHO cells were **labelled** with octopeptide 4Rh (FIG. 4A; 37.degree. C.). A gradual increase in the mean fluorescence signal of the cell population was observed as a function of time. **Monitoring** the mean fluorescence intensity of CHO cells exposed to 4Rh at both 4.degree. C. and 37.degree. C. confirmed that peptide-membrane association and peptide import were two distinct steps (FIG. 4B). The fact that both events reached a plateau at later incubation periods could be rationalized by one of two possible import mechanisms: receptor-mediated endocytosis or by the broader but less specific mechanism of adsorptive endocytosis. The existence of a distinct class of receptors for octopeptide 4Rh can only be confirmed if one can demonstrate the saturability and reversibility of the interaction between 4Rh and such receptors. **Monitoring** the mean fluorescence signal of 4Rh upon addition of increasing concentrations of the octopeptide to CHO cells at both 4.degree. C. and 37.degree. C. suggested that both binding and uptake events are potentially saturable (FIG. 5). However, the addition of excess octopeptide 4 (non-fluorescent analog), to block or displace 4Rh bound to CHO cells at 4.degree. C. did not inhibit its association with these cells (results not shown). These results suggest the existence of low affinity/high occupancy sites for 4Rh on CHO cells reminiscent of a nonspecific uptake process. It is well known that poly-L-lysine associates with the negatively charged surface of mammalian cells and is internalized by a process termed nonspecific adsorptive endocytosis (Ryser and Shen, 1978; Shen and Ryser, 1979; Leonetti et al., 1990). Interestingly, members of the integrin family of cell adhesion molecules, particularly .alpha..sub.3 .beta..sub.1, .alpha..sub.5 .beta..sub.1 and .alpha..sub.6 .beta..sub.1 could serve as a transitory class of cell surface receptors for CTS domains (Vogel et al., 1993). Past studies have shown that inhibitors of ATP **synthesis**, such as 2-deoxyglucose (10 mM) and sodium azide (5 mM) could inhibit the uptake process of poly-lysine polymers (Leonetti et al., 1990). A weak, but reproducible, inhibition of octopeptides endocytosis into CHO cells in the presence of these reagents was observed (FIG. 6A). No nuclear localization was observed over that time period (FIG. 6B).

DETDESC:

DETD(79)

Field, G. B., and Noble, R. L. (1990). **Solid**-phase peptide **synthesis** utilizing 9-fluoromethoxycarbonyl amino acids. Int. J. Peptide Protein Res. 35, 161-214.

DETDESC:

DETD(103)

Tam, J. P. (1988). Synthetic peptide vaccine design: **synthesis** and properties of a high-density **multiple** antigenic peptide system. Proc. Natl. Acad. Sci. U.S.A. 85, 5409-5413.

6. 5,635,602, Jun. 3, 1997, Design and synthesis of bispecific DNA-antibody conjugates; Charles R. Cantor, et al., 530/391.1, 387.3, 391.5, 391.9; 536/23.1 [IMAGE AVAILABLE]

US PAT NO: 5,635,602 [IMAGE AVAILABLE] L7: 6 of 20

ABSTRACT:
The invention relates to bis-protein-DNA conjugates. A protein having an antigen specific binding activity is covalently linked to each end of a derivatized DNA molecule. The bis-protein-DNA conjugates can be used for immunoassays and measuring distances between proteins at up to 3.4 .ANG. resolution. The invention also relates to methods of synthesizing these bis-protein-DNA conjugates. Synthesis of the conjugates entails derivatizing the 5' or 3' end of a DNA oligonucleotide and covalently linking that DNA to a protein. The DNA can be indirectly conjugated to an antibody or Fab' fragment, using a avidin/streptavidin-biotin linkage. The conjugates of the invention can be used in immunoassays and PCR assays.

DETDESC:

DETD(4)

A special advantage of using DNA as a molecular scaffold for constructing **arrays** of other **molecules** is that one is not limited to DNA with 2 ends. For example, 3 and 4 ended junctions have been made and they can form the basis for an endless array of more complex structures. Therefore, tri- and tetra-specific antibodies can be prepared using other higher order DNA structures such as Holliday junctions in conjunction with the methods provided below for preparing bi-specific antibodies. In addition, numerous proteins are known with very specific DNA binding sites. Such proteins can augment and enhance the methods for starting with DNA and producing a specific assembly of other molecules.

DETDESC:

DETD(11)

In the initial attempts to **synthesize** DNA-antibody cross-links, a variety of direct chemical cross-linking agents proved unsuccessful. These studies were plagued by inefficient cross-linking (especially in the case of antibody Fab' fragments) difficulty in separating free DNA from products and other reactants by gel filtration, and loss of antibody-antigen binding activity. Use of streptavidin overcame these problems. That is, streptavidin as an intermediate cross-linking molecule between biotinylated antibody and biotinylated DNA provided several advantages: gentle and efficient conjugation; the possibility of **monitoring** the conjugation with dye-**labeled** reagents; and efficient purification through use of affinity chromatography with 2-iminobiotin followed by standard gel filtration. However, additional geometric uncertainty and molecular weight introduced by streptavidin complex could prove to be troublesome. The multi-valence of streptavidin may also be troublesome for some applications compared to the bivalence of many chemical cross-linkers since these conjugates are big entities. Helpful, though, is the fact that a variety of proteins can be gently biotinylated, sacrificing little biological activity.

DETDESC:

DETD(86)

Activated thiol derivatives were prepared essentially according to the method of Brennan et al. (1985). F(ab').sub.2 fragments, obtained by pepsin cleavage (Parham, 1983) of 1 to 5 mg of polyclonal affinity-purified goat anti-mouse IgG (1 mg), monoclonal mouse IgG1 anti-human MHC class I (GA2) (3 mg) or anti-human CD4 (anti-Leu3a) (4 mg) were incubated overnight at room temperature in buffer containing 0.1M sodium phosphate pH 6.8, 2 mM 2-mercaptoethylamine-HCl (Pierce Chemical Co.) and 1 mM EDTA with or without 10 mM sodium arsenite (for free thiol group protection) at a protein concentration of 3 mg/ml. The next day, excess **solid** 5'dithobis(2-nitrobenzoic acid) (Ellman's reagent) was added to the solutions to a concentration of 10 mM. After incubation for 3 h at 25.degree. C., the reaction solutions were desalted and exchanged into buffer containing 0.1M sodium phosphate pH 6.8 and 1 mM EDTA using 6,000 molecular weight exclusion polyacrylamide columns. Five mg of mouse IgG2a F(ab').sub.2 anti-human TCR idiotype (T40/25) on HPB-ALL cells, was reduced to Fab' at 37.degree. C. by treatment for 90 min with 1 ml of 50 mM 2-mercaptoethylamine.HCl, 5 mM EDTA, pH 6.0 (according to the manufacturer's instructions, Pierce Chemical Co.). The sample was then desalted, reacted with Ellman's reagent (added as a **solid** to 10 mM) for 3 hr. at 25.degree. C., and then desalted again into 0.1M sodium phosphate pH 6.8 and 1 mM EDTA as above. In addition, some derivatives were **synthesized** using FITC- and TRITC-conjugated F(ab').sub.2 as the starting material. The dye molecules were usually attached via .epsilon.-amino groups of free lysine residues, and thus would not interfere with these disulfide exchange reactions.

DETDESC:

DETD(89)

Purified, complementary 5'-end-thiolated oligonucleotides (SEQ ID NOS: 1 and 2) were separately mixed with equimolar amounts of thionitrobenzoate-derivatized antibody fragments and allowed to react separately for 16 h at room temperature. If crude derivatized oligonucleotide (purity varied from 70 to 90%) was used, the reaction was carried out in two-fold molar DNA excess. The concentration of Fab' in the reaction solution was approximately 1 to 2 mg/ml. Released thionitrobenzoate, as a consequence of disulfide exchange, could be **monitored** by absorbance at 412 nm (.epsilon..sub.412 =1.36.times.10.sup.4 cm.sup.-1.M.sup.-1) and observed as a yellow color in the reaction solution. The reaction mixtures were then exchanged into 20 mM TrisCl and 1 mM EDTA and purified by anion exchange HPLC (7.5 cm.times.7.5 mm DEAE-3SW, Toso Haas) at a flow rate of 1 ml/min with elution by a 0-800 mM NaCl gradient. Fractions were analyzed with 7.5% SDS-PAGE (Laemmli, 1970) and silver staining (Biorad). Purified Fab'-single-stranded DNA conjugates were then mixed and allowed to anneal at 0.degree. C. SDS-PAGE gels were also stained with ethidium bromide (unfixed) and Coomassie (fixed) stains. To confirm the presence of DNA in putative conjugate fractions, treatments with DNHAse I (Sigma Chemical

Co.) were performed. Non-denaturing PAGE analysis (Davis, 1964) was also performed on single and double-stranded conjugates. The entire **synthetic** scheme is diagrammed in FIG. 8. Dye **labeling**, which is not illustrated here, may in many cases only be done after Fab'-DNA conjugation, as dye **labeling** introduces significant heterogeneity in both the reaction and the separation (unpublished observations). Dye-**labeled** antibody preparations must be purified and characterized before carrying out such conjugation reactions.

DETDESC:

DETD(198)

Huse, W. D., Sastry, L., Iverson, S. A., Kang, A. S., Alting-Mees, M., Burton, D. R., Benkovic, S. J., and Lerner, R. A. (1989). Generation of a large **combinatorial** **library** of the immunoglobulin repertoire in phage lambda. Science 246, 1275-1281.

7. 5,614,402, Mar. 25, 1997, 5' nucleases derived from thermostable DNA polymerase; James E. Dahlberg, et al., 435/199, 194 [IMAGE AVAILABLE]

US PAT NO: 5,614,402 [IMAGE AVAILABLE] L7: 7 of 20

ABSTRACT:

A means cleaving a nucleic acid cleavage structure in a site-specific manner is disclosed. A cleaving enzyme having 5' nuclease activity without interfering nucleic acid synthetic ability is employed as the basis of a novel method of detection of specific nucleic acid sequences.

SUMMARY:

BSUM(12)

The immobilization of target nucleic acids to solid surfaces such as nylon or nitrocellulose is a common practice in molecular biology. Immobilization formats eliminate the reassociation problem that can occur between complementary strands of target molecules, but not the problems associated with secondary structure effects. However, these mixed phase formats (i.e., Southern hybridization or dot blot hybridization) require time consuming fixation procedures. The hybridization reaction itself is kinetically much slower than a solution phase hybridization reaction. Together, the fixation and hybridization procedures require a minimum of several hours to several days to perform. Additionally, the standard immobilization procedures are often inefficient and result in the attachment of many of the target **molecules** to **multiple** portions on the solid surface, rendering them incapable of subsequent hybridization to probe molecules. Overall, these combined effects result in just a few percent of the initial target molecules being bound by probes in a hybridization reaction.

DETDESC:

DETD(85)

The following is an example of the dual capture assay to detect an antigen(s): A sample to be analyzed for a given antigen(s) is provided. This sample may comprise a mixture of cells; for example, cells infected with viruses display virally-encoded antigens on their surface. If the antigen(s) to be detected are present in solution, they are first attached to a **solid** **support** such as the wall of a microtiter dish or to a bead using conventional methodologies. The sample is then mixed with 1) the **synthetic** domain of a thermostable DNA polymerase conjugated to an antibody which recognizes either a first antigen or a first epitope on an antigen, and 2) the 5' nuclease domain of a thermostable DNA polymerase conjugated to a second antibody which recognizes either a second, distinct antigen or a second epitope on the same antigen as recognized by the antibody conjugated to the **synthetic** domain. Following an appropriate period to allow the interaction of the antibodies with their cognate antigens (conditions will vary depending upon the antibodies used; appropriate conditions are well known in the art), the sample is then washed to remove unbound antibody-enzyme domain complexes. dATP, dTTP and a small amount of poly d(A-T) is then added to the washed sample and the sample is incubated at elevated temperatures (generally in the range of 60.degree.-80.degree. C. and more preferably, 70-75.degree. C.) to permit the thermostable **synthetic** and 5' nuclease domains to function. If the sample contains the antigen(s) recognized by both separately conjugated domains of the polymerase, then an exponential increase in poly d(A-T) production occurs. If only the antibody conjugated to the **synthetic** domain of the polymerase is present in the sample such that no 5' nuclease domain is present in the washed sample, then only an arithmetic increase in poly d(A-T) is possible. The reaction conditions may be controlled in such a way so that an arithmetic increase in poly d(A-T) is below the threshold of detection. This may be accomplished by controlling the length of time the reaction is allowed to proceed or by adding so little poly d(A-T) to act as template that in the absence of nuclease activity to generate new poly d(A-T) primers very little poly d(A-T) is **synthesized**.

DETDESC:

DETD(88)

The production of poly d(A-T) may be detected in many ways including: 1) use of a radioactive **label** on either the dATP or dTTP supplied for the **synthesis** of the poly d(A-T), followed by size separation of the reaction products and autoradiography; 2) use of a fluorescent probe on the dATP and a biotinylated probe on the dTTP supplied for the **synthesis** of the poly d(A-T), followed by passage of the reaction products over an avidin bead, such as magnetic beads conjugated to avidin; the presence of the florescent probe on the avidin-containing bead indicates that poly d(A-T) has been formed as the fluorescent probe will stick to the avidin bead only if the fluorescenated dATP is incorporated into a covalent linkage with the biotinylated dTTP; and 3) changes fluorescence polarization indicating an increase in size. Other means of detecting the presence of poly d(A-T) include the use of intercalating fluorescence indicators to **monitor** the increase in duplex DNA formation.

DETDESC:

DETD(117)

To determine whether other 5' nucleases in other DNAPs would be suitable for the present invention, an **array** of enzymes, several of which were reported in the literature to be free of apparent 5' nuclease activity, were examined. The ability of these other enzymes to cleave nucleic acids in a structure-specific manner was tested using the hairpin substrate shown in FIG. 6 under conditions reported to be optimal for **synthesis** by each enzyme.

8. 5,599,662, Feb. 4, 1997, Oliconucleotide primers and probes for the detection of HIV-1; Richard A. Respess, 435/5, 6, 91.2; 536/24.32, 24.33; 935/8, 17, 78 [IMAGE AVAILABLE]

US PAT NO: 5,599,662 [IMAGE AVAILABLE] L7: 8 of 20

ABSTRACT:

The present invention provides improved primers for the polymerase chain reaction (PCR) amplification of a nucleic acid sequence from the pol gene of the human immunodeficiency virus type 1 (HIV-1). The invention also provides improved probes for the detection of the nucleic acid amplified using the primers of the invention. The primers and amplification methods of the invention enable the detection of HIV-1 from any of the known subtypes. The probes of the invention enable simple and rapid hybridization detection assays for detecting amplified HIV-1 nucleic acid.

SUMMARY:

BSUM(18)

The exact size of an oligonucleotide depends on many factors and the ultimate function or use of the oligonucleotide. Oligonucleotides can be prepared by any suitable method, including, for example, cloning and restriction of appropriate sequences and direct chemical **synthesis** by a method such as the phosphotriester method of Narang et al., 1979, Meth. Enzymol. 68:90-99; the phosphodiester method of Brown et al., 1979, Meth. Enzymol. 68:109-151; the diethylphosphoramidite method of Beaucage et al., 1981, Tetrahedron Lett. 22:1859-1862; and the **solid** **support** method of U.S. Pat. No. 4,458,066, each incorporated herein by reference. A review of **synthesis** methods is provided in Goodchild, 1990, Bioconjugate Chemistry 1(3):165-187, incorporated herein by reference.

SUMMARY:

BSUM(64)

An alternative method for detecting the amplification of HIV-1 nucleic acid by **monitoring** the increase in the total amount of double-stranded DNA in the reaction mixture is described in Higuchi et al., 1992, Bio/Technology 10:413-417; Higuchi et al., 1993, Bio/Technology 11:1026-1030; and European Patent Publication Nos. 487,218 and 512,334, each incorporated herein by reference. The detection of double-stranded target DNA relies on the increased fluorescence that ethidium bromide (EtBr) and other DNA binding **labels** exhibit when bound to double-stranded DNA. The increase of double-stranded DNA resulting from the **synthesis** of target sequences results in a detectable increase in fluorescence. The primers of the present invention are particularly useful because they enable amplification with unusually low levels of background non-specific amplification products.

SUMMARY:

BSUM(65)

The present invention also relates to kits, multicontainer units comprising useful components for practicing the present method. A useful kit can contain primers for the PCR amplification of HIV-1 nucleic acid. A kit can also contain means for detecting amplified HIV-1 nucleic acid, such as oligonucleotide probes. In some cases, the probes are fixed to an appropriate **support** membrane. Other optional components of the kit include, for example, an agent to catalyze the **synthesis** of primer extension products, the substrate nucleoside triphosphates, means used to label (for example, an avidin-enzyme conjugate and enzyme substrate and chromogen if the label is biotin), the appropriate buffers for PCR or hybridization reactions, and instructions for carrying out the present method.

DETDESC:

DETD(36)

One hundred .mu.l of avidin-HRP conjugate (available from **multiple** sources, e.g., Fluka **Chemical** Corp., Ronkonkoma, N.Y., and Sigma Chemical Co., St. Louis, Mo.) were added to each well in the plate. The plate was covered and incubated 15 minutes at 37.degree. C. and again washed as described above. One hundred .mu.l of a chromogen solution containing tetramethylbenzadine (available from **multiple** sources, e.g., Fluka **Chemical** Corp., Ronkonkoma, N.Y., and Sigma Chemical Co., St. Louis, Mo.) and H.sub.2 O.sub.2 were added to each well of the plate. The plate was then covered and incubated in the dark for 10 minutes at room temperature (20.degree. C. to 25.degree. C.) to allow the color to develop. Finally, 100 .mu.l of Stop Reagent (5% H.sub.2 SO.sub.4) was added to each well. The absorbance of each well of 450 nM was read within one hour of adding the Stop Reagent.

9. 5,549,974, Aug. 27, 1996, Methods for the **solid** phase **synthesis** of thiazolidinones, metathiazanones, and derivatives thereof; Christopher P. Holmes, 428/403, 406, 407, 411.1, 426, 457;

544/54; 548/182 [IMAGE AVAILABLE]

US PAT NO: 5,549,974 [IMAGE AVAILABLE] L7: 9 of 20

ABSTRACT:
The invention provides an efficient and versatile method for the **combinatorial** **synthesis** and screening of **libraries** of 4-thiazolidinones, metathiazanones, and derivatives thereof. In order to expediently **synthesize** a **combinatorial** **library** of derivatives based upon these core structures, a general methodology for the **solid** phase **synthesis** of these derivatives is also provided. **Arrays** of thiazolidinones, metathiazanones, and derivatives thereof useful as peptidomimetics and for the identification of agents having antifungal, antihistaminic, or antimicrobial activity or use in the treatment of inflammation, hypertension, renal failure, congestive heart failure, uremia and other conditions can be prepared using this method.

TITLE: Methods for the **solid** phase **synthesis** of thiazolidinones, metathiazanones, and derivatives thereof

ABSTRACT:
The invention provides an efficient and versatile method for the **combinatorial** **synthesis** and screening of **libraries** of 4-thiazolidinones, metathiazanones, and derivatives thereof. In order to expediently **synthesize** a **combinatorial** **library** of derivatives based upon these core structures, a general methodology for the **solid** phase **synthesis** of these derivatives is also provided. **Arrays** of thiazolidinones, metathiazanones, and derivatives thereof useful as peptidomimetics and for the identification of agents having antifungal, antihistaminic, or antimicrobial activity or use in the treatment of inflammation, hypertension, renal failure, congestive heart failure, uremia and other conditions can be prepared using this method.

SUMMARY:

BSUM(2)

The present invention is related to the area of chemical **synthesis**. More specifically, one embodiment of the present invention provides methods for the **solid** phase and **combinatorial** **synthesis** of 4-thiazolidinones, metathiazanones and derivatives thereof.

SUMMARY:

BSUM(3)

Obtaining a better understanding of the important factors in molecular recognition in conjunction with developing potent new therapeutic agents is a major focus of scientific research. Chemical and biological methods have recently been developed for the generation of large **combinatorial** **libraries** of peptides and oligonucleotides that are then screened against a specific receptor or enzyme in order to determine the key molecular recognition elements of the biopolymer for that receptor or enzyme. See U.S. Pat. No. 5,143,854; Ser. No. 07/805,727, filed Dec. 6, 1991, now U.S. Pat. No. 5,424,186; Ser. No. 07/624,120, filed Dec. 6, 1990, now abandoned; Ser. No. 07/946,239, filed Sep. 16, 1992, still pending; Ser. No. 07/762,522, filed Sep. 18, 1991, now abandoned; Ser. No. 07/978,940, filed Nov. 19, 1992, now abandoned; and Ser. No. 07/971,181, filed Nov. 2, 1992, now abandoned; each of which is assigned to the assignee of the present invention and incorporated herein by reference for all purposes. These methods provide rapid and efficient means to synthesize polymers that are biocompatible, i.e., compounds that are non-toxic and readily absorbed, and ideally are synthesized from monomers available in large quantity, with a reasonable shelf life, optical activity, high-fidelity coupling chemistry, and stable to various chemical reagents used for protecting and deprotecting various side chains.

SUMMARY:

BSUM(4)

Virtually any bioavailable organic compound can be accessed by chemical synthesis; however, such compounds typically are still synthesized and evaluated one at a time in many cases, thus dramatically limiting the number of derivatives which can be studied. This limitation can be overcome by developing the methodology for the **combinatorial** **synthesis** of large numbers of derivatives of therapeutically important classes of bioavailable organic compounds. Screening these compounds against key receptors or enzymes would then greatly accelerate the acquisition of useful structure versus recognition data and would revolutionize the search for potent new therapeutic agents.

SUMMARY:

BSUM(5)

The search for suitable small organic **molecules** amenable to a **combinatorial** **synthesis** approach is an ongoing quest. One ideal goal is to tailor the chemistry used to assemble the molecules to work in a polymer-supported fashion, in analogy to **solid** phase techniques commonly employed for peptides and oligonucleotides. The advantages of such a goal is twofold: not only does one gain overall efficiency through the ability to filter away both byproducts and excess reagents, but one also raises the possibility of mass screening of the immobilized molecules with techniques such as VLSIPS.TM. and ESL technologies. See, U.S. Pat. No. 5,143,854; Ser. No. 07/805,727, filed Dec. 6, 1991, U.S. Pat. No. 5,424,186; Ser. No. 07/624,120, filed Dec. 6, 1990, now abandoned; Ser. No. 07/946,239, filed Sep. 16, 1992, still pending; and Ser. No. 07/762,522, filed Sep. 18, 1991, now abandoned; each of which is assigned to the assignee of the present invention and incorporated herein by reference for all purposes.

SUMMARY:

BSUM(6)

Perhaps the first example of the application of **combinatorial** organic **synthesis** to non-polymeric organic compounds can be found in the work of Ellman who described the **solid** phase **synthesis** of a 1,4-benzodiazepines. See U.S. Pat. No. 5,288,514, which is incorporated herein by reference for all purposes. The benzodiazepines were **synthesized** on a **solid** **support** by the connection of three building blocks: an amino benzophenone; an amino acid; and an alkylating agent.

SUMMARY:

BSUM(7)

Hobbs Dewitt has reported on the generation of **libraries** of small **molecules**, which she terms "diversomers". Target compounds, including dipeptides, hydantoins, and benzodiazepenes, were **synthesized** simultaneously but separately, on a **solid** **support** in an **array** format, to generate a collection of up to 40 discrete structurally related compounds. The key step in this strategy involves the revealing of distal functionality which initiates attack on the bond linking the compound to the resin, thus, releasing the product from the resin.

SUMMARY:

BSUM(13)

The invention provides a rapid approach for **combinatorial** **synthesis** and screening of **libraries** of 4-thiazolidinones, metathiazonones, and derivatives thereof which overcomes the above-described limitations of current methodologies.

SUMMARY:

BSUM(14)

In one aspect, the present invention provides a method for the **solid** phase **synthesis** of thiazolidinones, metathiazonones, and derivatives thereof, which method includes the steps of first coupling an amine component to a **solid** **support** and then treating the immobilized amine component with a carbonyl component and a thiol component. In another aspect, the present invention provides a method for the **solid** state **synthesis** of thiazolidinones, metathiazonone, and derivatives thereof, which method includes the steps of first coupling a thiol component to a **solid** **support** and treating the immobilized thiol component with an amine component and a carbonyl component.

DRAWING DESC:

DRWD(3)

FIG. 2 illustrates the use of .sup.13 C NMR to **monitor** the stability of thiazolidinones. Panel C shows the .sup.13 C NMR spectrum of **support**-bound thiazolidinone which has been doubly **labeled** with a .sup.13 C-atom at the position 2 of the ring and at the position alpha to the carbonyl of the linker (**labeled** positions are indicated with a "*"). Panel B shows the .sup.13 C NMR spectrum of **support**-bound doubly **labeled** thiazolidinone after treatment with 95% TFA for one hour. Panel A shows the .sup.13 C NMR spectrum of **support**-bound doubly **labeled** thiazolidinone after 40 cycles of DNA **synthesis**.

DRAWING DESC:

DRWD(5)

FIG. 4 shows an HLPC trace for the reaction mixture produced by subjecting a **support**-bound thiazolidinone to 40 cycles of DNA **synthesis** and 3 hour photolysis in PBS buffer.

DRAWING DESC:

DRWD(9)

FIG. 8 shows HPLC traces for the products of a solution preparation and a **solid** state **synthesis** of a thiazolidinone prepared from glycine, mercaptoacetic acid, and 3-pyridinecarboxaldehyde.

DRAWING DESC:

DRWD(10)

FIG. 9 shows HPLC traces for the products of a solution preparation and a **solid** state **synthesis** of a thiazolidinone prepared from glycine, thiolactic acid, and benzaldehyde.

DRAWING DESC:

DRWD(11)

FIG. 10 shows HPLC traces for the products of a solution preparation and a **solid** state **synthesis** of a thiazolidinone prepared from alanine, mercaptoacetic acid, and benzaldehyde.

DRAWING DESC:

DRWD(12)

FIG. 11 shows HPLC traces for the products of a solution preparation and a **solid** state **synthesis** of a metathiazanone prepared from glycine, .beta.-mercaptopropionic acid, and benzaldehyde.

DETDESC:

DETD(50)

"**Chemical** **library**" or "**array**" is an intentionally created **collection** of differing **molecules** which can be prepared either synthetically or biosynthetically and screened for biological activity in a variety of different formats (e.g., **libraries** of soluble **molecules**; and **libraries** of compounds tethered to resin beads, silica chips, or other solid supports). The term is also intended to refer to an intentionally created collection of stereoisomers.

DETDESC:

DETD(51)

"**Combinatorial** **synthesis** strategy" or "**combinatorial** **chemistry**" refers to an ordered strategy for the parallel synthesis of diverse compounds by sequential addition of reagents which leads to the generation of large **chemical** **libraries**. Thus, **combinatorial** **chemistry** refers to the systematic and repetitive, covalent connection of a set of different "building blocks" of varying structures to each other to yield large arrays of diverse molecular entities.

DETDESC:

DETD(54)

"Identifier tag" denotes a physical attribute that provides a means whereby one can identify a chemical reaction. The identifier tag serves to record a step in a series of reactions used in the **synthesis** of a **chemical** **library**. The identifier tag may have any recognizable feature, including for example: a microscopically or otherwise distinguishable shape, size, mass, color, optical density, etc.; a differential absorbance or emission of light; chemical reactivity; magnetic or electronic properties; or any other distinctive mark capable of encoding the required information, and decipherable at the level of one (or a few) molecules. A preferred example of such an identifier tag is an oligonucleotide, because the nucleotide sequence of an oligonucleotide is a robust form of encoded information. Identifier tags can be coupled to the **solid** **support**. Alternatively, the "identifier tag" can be coupled directly to the compound being **synthesized**, whether or not a **solid** **support** is used in the **synthesis**. In the latter embodiment, the identifier tag can conceptually be viewed as also serving as the "**support**" for **synthesis**.

DETDESC:

DETD(56)

"Linker" refers to a molecule or group of molecules attached to a **solid** **support** and spacing a **synthesized** compound from the **solid** **support**, such as for exposure/binding to a receptor.

DETDESC:

DETD(57)

"Predefined region" refers to a localized area on a **solid** **support** which is, was, or is intended to be used for formation of a selected molecule and is otherwise referred to herein in the alternative as a "selected" region. The predefined region may have any convenient shape, e.g., circular, rectangular, elliptical, wedge-shaped, etc. For the sake of brevity herein, "predefined regions" are sometimes referred to simply as "regions." In some embodiments, a predefined region and, therefore, the area upon which each distinct compound is **synthesized** is smaller than about 1 cm.sup.2 or less than 1 mm.sup.2. Within these regions, the molecule **synthesized** therein is preferably **synthesized** in a substantially pure form. In additional embodiments, a predefined region can be achieved by physically separating the regions (i.e., beads, resins, gels, etc.) into wells, trays, etc.

DETDESC:

DETD(58)

"Protecting group" refers to a chemical group that exhibits the following characteristics: (1) reacts selectively with the desired functionality in good yield to give a derivative that is stable to the projected reactions for which protection is desired; 2) can be selectively removed from the derivatized **solid** **support** to yield the desired functionality; and 3) is removable in good yield by reagents compatible with the other functional group(s) generated in such projected reactions. Examples of protecting groups can be found in Greene et al. (1991) Protective Groups in Organic **Synthesis**, 2nd Ed. (John Wiley & Sons, Inc., New York). Preferred protecting groups include photolabile protecting groups (such as methylnitropiperonyloxycarbonyl (Menpoc), methylnitropiperonyl (Menp), nitroveratryl (Nv), nitroveratryloxycarbonyl (Nvoc), or nitroveratryloxymethyl ether (Nvom)); acid-labile protecting group (such as Boc or DMT); base-labile protecting groups (such as Fmoc, Fm, phosphonioethoxycarbonyl (Peoc, see Kunz (1976) Chem. Ber. 109:2670); groups which may be removed under neutral conditions (e.g., metal ion-assisted hydrolysis), such as DBMB (see Chattopadhyaya et al. (1979) J.C.S. Chem. Comm. 987-990), allyl or alloc (see, e.g., Greene and Wuts, "Protective Groups in Organic **Synthesis**", 2nd Ed., John Wiley & Sons, Inc., New York, N.Y. (1991), 2-haloethyl (see Kunz and Buchholz (1981) Angew. Chem. Int. Ed. Engl. 20:894), and groups which may be removed using fluoride ion, such as 2-(trimethylsilyl)ethoxymethyl (SEM), 2-(trimethylsilyl)ethyloxycarbonyl (Teoc) or 2-(trimethylsilyl)ethyl (Te) (see, e.g., Lipshutz et al. (1980) Tetrahedron Lett. 21:3343-3346)); and groups which may be removed under mild reducing conditions (e.g., with sodium borohydride or hydrazine), such as Lev. Id. at 30-31, 97, and 112. Particularly preferred protecting groups include Fmoc, Fm, Menpoc, Nvoc, Nv, Boc, CBZ, allyl, alloc, Npeoc (4-nitrophenethyloxycarbonyl) and Npeom (4-nitrophenethyloxy-methyloxy).

DETDESC:

DETD(59)

"**Solid** **support**" or "**support**" refers to a material or group of materials having a rigid or semi-rigid surface or surfaces. In many embodiments, at least one surface of the **solid** **support** will be substantially flat, although in some embodiments it may be desirable to physically separate **synthesis** regions for different compounds with, for example, wells, raised regions, pins, etched trenches, or the like. According to other embodiments, the **solid** **support**(s) will take the form of beads, resins, gels, microspheres, or other geometric configurations. The **solid** **support** is alternatively referred to herein as a **support**.

DETDESC:

DETD(82)

The present invention, in one aspect, includes a highly efficient and versatile method of synthesizing and screening, preferably in parallel and simultaneous fashion, large numbers of 4-thiazolidinones, metathiazanones and derivatives thereof. Thus, according to one aspect, the present invention provides a **solid**-phase **synthesis** method for 4-thiazolidinones, metathiazanones, and derivatives thereof in which variable substituent groups are attached to a common central structure. This **solid**-phase **synthesis** permits each reaction to be confined to a predefined region of a small **solid** structure. The physical joining of a multitude of small **solid** structures into a single unit, for example, then permits the simultaneous handling of a multitude of compounds and reagents. The use of structures of this kind for certain multiple simultaneous reactions is known in the art, and its application to the present invention will become apparent from the description which follows.

DETDESC: .

DETD(83)

In order to expediently **synthesize** a **combinatorial** **library** of 4-thiazolidinones, metathiazanones, and derivatives thereof, a generalized methodology for the **solid** phase **synthesis** of these compounds is also provided. **Synthesis** on **solid** **support** proceeds in sufficiently high yield in preferred embodiments such that purification and isolation steps can be eliminated and thus, dramatically increasing **synthesis** efficiency. According to one embodiment, the method of synthesizing 4-thiazolidinones, metathiazanones, and derivatives thereof comprises the steps of first binding an amine component to a **solid** **support**. Preferably, the amine component will comprise a primary amine, and more preferably, an amino acid, a peptide, a mono-substituted hydrazine derivative or a hydrazide derivative. The heterocycle is then formed by treating the **solid** **support**-bound amine component, either sequentially or simultaneously, with a carbonyl component, preferably an aldehyde, and a thiol component, preferably an .alpha.--mercapto carboxylic acid or a .beta.-mercapto carboxylic acid. According to another embodiment, the thiol component is immobilized on the **support** and the heterocycle is formed by treatment of the immobilized component with a carbonyl component, preferably an aldehyde, and an amine component, preferably an .alpha.-amino acid, a peptide, a monoprotected or mono-substituted hydrazine derivative or a hydrazide derivative.

DETDESC:

DETD(87)

"**Solid** **support**" embraces a particle with appropriate sites for oligomer **synthesis** and, in some embodiments, tag attachment and/or **synthesis**. **Solid** supports may consist of many materials, limited primarily by capacity for derivatization to attach any of a number of chemically reactive groups and compatibility with the **synthetic** **chemistry** used to produce the **array** and, in some embodiments the methods used for tag attachment and/or **synthesis**. Suitable **support** materials typically will be the type of material commonly used in peptide and polymer **synthesis** and include glass, latex, heavily cross-linked polystyrene or similar polymers, gold or other colloidal metal particles, and other materials known to those skilled in the art. Except as otherwise noted, the chemically reactive groups with which such **solid** supports may be derivatized are those commonly used for **solid** phase **synthesis** of the polymer and thus will be well known to those skilled in the art, i.e., carboxyls, amines and hydroxyls.

DETDESC:

DETD(88)

To improve washing efficiencies, one can employ nonporous supports or other **solid** supports less porous than typical peptide **synthesis** supports; however, for certain applications of the invention, quite porous beads, resins, or other supports work well and are often preferable. A preferred **support** is glass, as described in U.S. Pat. No. 5,143,854, supra. Another preferred **solid** **support** is resin, such as the beads described in co-pending U.S. patent application Ser. No. 07/946,239, filed Sep. 16, 1992, supra. In general, the bead size is in the range of 1 nm to 100 .mu.m, but a more massive **solid** **support** of up to 1 mm in size may sometimes be used. Particularly preferred resins include Sasrin resin (a polystyrene resin available from Bachem Bioscience, Switzerland); and TentaGel S AC, TentaGel PHB, or TentaGel S NH.sub.2 resin (polystyrene-polyethylene glycol copolymer resins available from Rappe Polymere, Tubingen, Germany). Other preferred

supports are commercially available and described by Novabiochem, La Jolla, Calif.

DETDESC:

DETD(90)

When bound to a **solid** **support**, the thiazolidinone and any associated tags are usually attached by means of one or more molecular linkers. The linker molecules preferably have lengths sufficient to allow the compounds to which they are bound to interact freely with any molecules exposed to the **solid** **support** surface, such as **synthetic** reagents or receptors which are an object of study. The linker molecule, prior to attachment, has an appropriate functional group at each end, one group appropriate for attachment to the **support** and the other group appropriate for attachment to the thiazolidinone or tag.

DETDESC:

DETD(91)

One can, of course, incorporate a wide variety of linkers, depending upon the application and the effect desired. For instance, one can select linkers that impart hydrophobicity, hydrophilicity, or steric bulk to achieve desired effects on properties such as coupling or binding efficiency. In one aspect of the invention, branched linkers, i.e., linkers with bulky side chains such as the linker, Fmoc-Thr(tBu), are used to provide rigidity to or to control spacing of the **molecules** on the solid support in a **library** or between a **molecule** and a tag in the **library**. In some embodiments, cleavable linkers will be used to facilitate an assay or detection step as discussed more fully below.

DETDESC:

DETD(93)

The choice of functionality used for binding a molecule to the **solid** **support** will depend on the nature of the compound to be **synthesized** and the type of **solid** **support**. Conditions for coupling monomers and polymers to **solid** supports through a wide variety of functional groups are known in the art. See, e.g., U.S. Pat. Nos. 4,542,102; 4,282,287; Merrifield, "**Solid** Phase Peptide **Synthesis**," J. Am. Chem. Soc., (1963) 85:2149-2154; Geysen et al., "Strategies for Epitope Analysis Using Peptide **Synthesis**," J. Imm. Meth., (1987) 102:259-274; Pirrung et at., U.S. Pat. No. 5,143,854; and Fodor et al., "Light-Directed Spatially-Addressable Parallel Chemical **Synthesis**," Science (1991) 251:767-773, each of which is incorporated herein by reference.

DETDESC:

DETD(121)

The active sites of the surface and/or the immobilized component are optionally protected initially by protecting groups which may be acid-, base-, or photoremovable protecting groups as discussed above. Among a wide variety of protecting groups are materials such as FMOC, BOC, t-butyl esters, t-butyl ethers, and the like. Various exemplary protecting groups are described in, for example, Atherton et al., **Solid** Phase Peptide **Synthesis**, IRL Press (1989), incorporated herein by reference and U.S. Pat. No. 5,148,854.

DETDESC:

DETD(141)

For some applications, one may desire a "**support**-free" or "soluble" **library** of **molecules**. Soluble **molecules**, both tagged and untagged, can be useful for a variety of purposes, including assaying the activity of a compound and structural analysis. The generation of soluble molecular libraries, both tagged and untagged, and the solubilization of compounds, both tagged and untagged, **synthesized** on a **solid** **support** can be accomplished by techniques known in the art.

DETDESC:

DETD(142)

Typically, cleavable linkers such as those described in U.S. Ser. No. 978,940, filed Nov. 19, 1992, incorporated herein by reference can be employed. To produce a soluble tagged molecule, the cleavable linker will be attached to the bead or other **solid** **support** and have at least two functional groups: one for synthesizing the molecule of interest and the other for synthesizing the tag. Thus, the molecule and tag can be **synthesized** attached to a common linker, which, in turn, is bound to the **solid** **support**. Once the molecule and tag are **synthesized**, the linker is cleaved to provide a soluble tagged molecule

DETDESC:

DETD(152)

A further example of the use of .sup.13 C NMR to perform stability studies is shown in FIGS. 2 and 3. Specifically, Panel C of FIG. 2 (or FIG. 3) shows a .sup.13 C NMR spectrum of a **support**-bound thiazolidinone with a sharp peak at about 43 ppm attributable to the labeled carbon. As shown in Panels A and B of FIG. 2, additional resonances did not appear when the **support**-bound thiazolidinone was subjected to acid treatment, of the conditions typically used in DNA **synthesis** (e.g., the conditions used to introduce an oligonucleotide tag, that is, alternating rounds of mild acid treatment, exposure to phosphoramidites, and mild oxidation conditions), thus demonstrating that thiazolidinones are stable to these conditions. See also, FIGS. 4 and 6 and discussion below.

DETDESC:

DETD(153)

Photolysis afforded cleavage of the thiazolidinone from the **support**. Further photolysis did not effect the .sup.13 C resonance. See Panels A and B of FIG. 3. The **support**-bound thiazolidinone which had been subjected to 40 cycles of DNA **synthesis** was also cleaved from the **support** and analyzed by HPLC. Significantly, the HPLC trace showed the presence of the correct thiazolidinone product. See FIG. 4.

DETDESC:

DETD(161)

Thereafter, a first portion of a molecule to be **synthesized** is added to the **support**, for example, the first portion will typically be a substituted amino acid linked to the **support** via the carboxyl group. The amino group and the side chain functionality of the amino acid may be protected by an appropriate protecting group. Alternatively, the first portion will comprise the thiol component. The mercapto group and the carboxyl group may be protected with appropriate protecting groups. In other embodiments, the first portion will comprise the carbonyl component. The protecting group(s), if any, on the immobilized component may be the same as or different from the protecting group on the **solid** **support**. In most cases, the various regions will be coupled to different molecules.

DETDESC:

DETD(163)

The **synthesis** of **combinatorial** **chemistry** **libraries** can be characterized in terms of complexity. For example, as discussed above, the "pure" stepwise procedure using a plurality of amine components, a plurality of thiol components, and a plurality of carbonyl components in each coupling reaction with mixing and apportioning steps between each component addition generates all possible compounds in the fewest number of steps.

DETDESC:

DETD(175)

According to some embodiments, the **solid** **support** will bear an identifier tag. The identifier tag has a recognizable feature that is, for example, microscopically or otherwise distinguishable in shape, size, mass, charge, or color. This recognizable feature may arise from the optical, chemical, electronic, or magnetic properties of the tag, or from some combination of such properties. In essence, the tag serves to label a molecule and to encode information decipherable at the level of one (or a few) molecules or **solid** supports. By using identifier tags to track the **synthesis** pathway that each member of a **chemical** **library** has taken, one can deduce the structure of any **chemical** in the **library** by reading the identifier tag. Particularly preferred identifier tags include **synthetic** oligodeoxyribonucleotides. For further detail on identifier tags, see U.S. patent application Ser. No. 08/146,886 and Ser. No. 08,149,675. An example of a parallel **synthesis** of a thiazolidinone with an oligonucleotide tag is shown in FIG. 6 and described further below.

DETDESC:

DETD(176)

The identifier tags identify each reaction step that an individual library member or **solid** **support** has experienced and record the step in the **synthesis** series in which each chemical reaction was performed. The tags may be added immediately before, during, or after the chemical reaction, as convenient and compatible with the type of identifier tag, modes of attachment, and chemistry of molecular **synthesis**.

DETDESC:

DETD(177)

As shown herein, thiazolidinones are stable to light, DNA **synthesis**, and treatment of TFA. In addition, by varying the substituents, a set of compounds having unique molecular weights can be designed. Thus, the thiazolidinone, metathiazanone, or derivative thereof can find utility as an identifier tag. After cleavage, the tags can be analyzed using mass spectroscopy or other means of physical characterization. As described more fully below, techniques are available for cleaving just a portion of the **support**-bound molecules from the **support** while leaving the remainder of the **support**-bound molecules intact. Thus, thiazolidinones can serve as tags to identify other small molecules (e.g., diketopiperazines, pyrollidines, benzodiazepines, and the like), peptides or DNAs.

DETDESC:

DETD(181)

In a similar manner to that described above, the **solid** **support** is then exposed to a receptor of interest that is appropriately labeled with, or coupled to another receptor with a label, such as a fluorescent or radioactive label. The **solid** **support** is then scanned to determine the location of the label. From knowledge of the composition of the molecule **synthesized** at each site, it becomes possible to identify the molecule(s) that are complementary to the receptor.

DETDESC:

DETD(183)

Arrays also can be prepared using the pin approach developed by Geysen et al., for **combinatorial** **solid**-phase peptide **synthesis**. A description of this method is offered by Geysen et al., J. Immunol. Meth. (1987) 102:259-274, incorporated herein by reference. According to this method as it may be practiced in the present invention, a series of 96 pins are mounted on a block in an arrangement and spacing which correspond to a 96-well Microtiter reaction plate, and the surface of each pin is derivatized to contain terminal aminomethyl groups. The pin block is then lowered over a series of reaction plates in sequence to immerse the pins in the wells of the plates where coupling occurs at the terminal aminomethyl groups and the various reactions in the reaction schemes described above are performed.

DETDESC:

DETD(187)

An apparatus capable of preparing arrays of 4-thiazolidinones, metathiazonones, and derivatives thereof is described in U.S. patent application Ser. No. 08/149,675, filed Nov. 2, 1993, incorporated herein by reference. Such an instrument is capable of performing up to 100 or more parallel reactions simultaneously by distributing the reaction mixture or slurry of **synthesis** **solid** supports, under programmable control, to the various channels for pooling, mixing, and redistribution.

DETDESC:

DETD(188)

Another apparatus capable of preparing arrays according to the methods described herein is described in association with the **synthesis** of peptides in Geysen et al., J. Immun. Methods (1987) 102:259-274, incorporated herein by reference for all purposes. In brief, this method utilizes a **solid** **support** having a plurality of pins or other extensions. The pins are each inserted simultaneously into individual reagent containers in tray. Although in a common embodiment, an **array** of 96 pins/containers is utilized, it will be recognized that in other embodiments a larger **array** of such pins/containers will be provided. Each tray is filled with a particular reagent for coupling in a particular chemical reaction on an individual pin. Accordingly, the trays will often contain different reagents. Since the chemistry disclosed herein has been established such that a relatively similar set of reaction conditions may be utilized to perform each of the reactions, it becomes possible to conduct **multiple** **chemical** coupling steps simultaneously.

DETDESC:

DETD(196)

Since a wide **array** of substituted amino acids, aldehydes, and thiol components are readily available, the **synthesis** technique herein results in an **array** of immobilized materials which are at known locations on the **solid** **support** or in a soluble format and may be effectively used in screening studies to determine which of the **synthesized** materials show significant affinity for a receptor or receptors of interest. Receptor affinity can be studied by exposing the **solid** **support** to the receptor or receptors of interest, and determining where the receptor has bound to the **solid** **support**. In some embodiments, the location of the receptor on the **solid** **support** may be conveniently located by labeling the receptor with an radioactive or fluorescent label, and scanning the surface of the **solid** **support** for the presence of the receptor. In some embodiments, the receptor of interest may be unlabeled, but later exposed to a second receptor that is labeled and known to be complementary to the receptor of interest. The receptor will bind to the molecules that are complementary to the receptor while it will not bind to other molecules on the **solid** **support**. Accordingly, the present method provides an effective way to identify ligands that are complementary to a receptor.

DETDESC:

DETD(200)

Soluble molecules can also be screened using an immobilized receptor. After contacting the molecules with the immobilized receptor, and washing away non-specifically bound molecules, bound molecules are released from the receptor by any of a wide variety of methods. The tags, if present, are optionally amplified and then examined and decoded to identify the structure of the molecules that bind specifically to the receptor. A tagged molecule in solution can be assayed using a receptor immobilized by attachment to a bead, for example, by a competition assay with a fluorescently labeled ligand. The beads bearing immobilized receptors can be recovered and the sorted using FACS to identify positives (diminished fluorescence caused by the **library** **molecule** competing with the labeled ligand).

DETDESC:

DETD(201)

The soluble **molecules** of the **library** can be **synthesized** on a **solid** **support** and then cleaved prior to assay. In one embodiment, microscopic beads of a molecular library are placed in very small individual compartments or wells that have been "nanofabricated" in a silicon or other suitable surface. Beads are loaded into the wells by dispersing them in a volume of loading buffer sufficient to produce an average of one bead per well. In one embodiment, the solution of beads is placed in a reservoir above the wells and the beads are allowed to settle into the wells. Cleavage of the molecules from the beads may be accomplished using chemical or thermal systems, but a photocleavable system is preferred. The molecules of interest can be cleaved from the beads to produce either untagged molecules in solution (the tag remaining attached to the bad) or tagged molecules in solution. In either event, the molecules of interest are cleaved from the beads but remain contained within the compartment along with the bead and the identifier tag(s).

DETDESC:

DETD(202)

In another embodiment, relatively large tagged beads, from which the molecules of interest are cleaved in a series of reactions, are used. In this method, the beads are 50 to 500 .mu.m in diameter, with capacities equivalent to 100 to 500 pmol of **molecule** per bead. The **library** is divided into about 100 pools, each containing about 100,000 beads. A certain percentage, about 25% of the molecule of interest is cleaved from the pool.

DETDESC:

DETD(213)

Solid Phase **Synthesis** of 4-Thiazolidinones ##STR14## R=H, Me R.sub.1 =H, Me, iPr, CH.sub.2 PH

DETDESC:

DETD(218)

The observed purities measured via HPLC analysis obtained from one-pot, three component condensation reactions using valine as the amine component, different resins and linkers are reported in Table 2 below. The **solid** phase **synthesis** of all the thiazolidinones represented in Table 1 was carried out using the methods described above.

10. 5,543,293, Aug. 6, 1996, DNA ligands of thrombin; Larry Gold, et al., 435/6, 91.2; 536/22.1 [IMAGE AVAILABLE]

US PAT NO: 5,543,293 [IMAGE AVAILABLE] L7: 10 of 20

ABSTRACT:
Methods are described for the identification of nucleic acid ligand solutions to thrombin. The present invention utilizes the SELEX (Systematic Evolution of Ligands for EXponential Enrichment) method for identfying and preparing DNA ligands to thrombin. Further included in the present invention are modified nucleotide sequences based on the sequences of the DNA ligands identified. The modified DNA ligands to thrombin exhibit increased in vivo stability.

SUMMARY:

BSUM(10)

Despite these known instances, the great majority of proteins and other cellular components are thought not to bind to nucleic acids under physiological conditions and such binding as may be observed is non-specific. Either the capacity of nucleic acids to bind other compounds is limited to the relatively few instances enumerated supra, or the chemical repertoire of the nucleic acids for specific binding is avoided (selected against) in the structures that occur naturally. The present invention is premised on the inventors' fundamental insight that nucleic acids as **chemical** compounds can form a virtually limitless **array** of shapes, sizes and configurations, and are capable of a far broader repertoire of binding and catalytic functions than those displayed in biological systems.

DETDESC:

DETD(37)

High affinity RNA ligands for thrombin were isolated by SELEX. **Random** RNA **molecules** used for the initial candidate mixture were generated by in vitro transcription from a 102 nucleotide double-stranded DNA template containing a random cassette 30 nucleotides (30N) long. A population of 10.sup.13 30N DNA templates were created by PCR, using a 5' primer containing the T7 promoter for in vitro transcription, and restriction sites in both the 5' and 3' primers for cloning.

DETDESC:

DETD(38)

The RNA concentration for each round of SELEX was approximately 2-4.times.10.sup.-7 M and concentrations of thrombin (Sigma, 1000 units) went from 1.0.times.10.sup.-6 in the 1st round to 4.8.times.10.sup.-7 in rounds 2 and 3 and 2.4.times.10.sup.-7 in rounds 4-12. The binding buffer for the RNA and protein was 100 mM NaCl, 50 mM Tris/Cl, pH 7.7, 1 mM DTT, and 1 mM MgCl.sub.2. Binding was for 5 minutes at 37.degree. C. in a total volume of 100 .mu.l in rounds 1-7 and 200 .mu.l in rounds 8-12. Each binding reaction was filtered through a pre-wetted (with 50 mM Tris/Cl, pH 7.7) nitrocellulose filter (2.5 cm Millipore, 0.45 .mu.M) in a Millipore filter binding apparatus, and immediately rinsed with 5 ml of the same buffer. The RNA was eluted from the filters in 400 .mu.l phenol (equilibrated with 0.1M NaoAc pH 5.2), 200 .mu.l freshly prepared 7M urea as described (Tuerk et al. (1990) J. Mol. Biol. 213:749-761. The RNA was precipitated with 20 .mu.g tRNA, and was used as a template for cDNA **synthesis**, followed by PCR and in vitro transcription to prepare RNA for the subsequent round. The RNA was radio-**labeled** with .sup.32 P-ATP in rounds 1-8 so that binding could be **monitored**. In order to expedite the time for each round of SELEX, the RNA was not **labeled** for rounds 9-12. RNA was prefiltered through nitrocellulose filters (1.3 cm Millipore, 0.45 .mu.M) before the 3rd, 4th, 5th, 8th, 11th, and 12th rounds to eliminate selection for any nonspecific nitrocellulose binding.

DETDESC:

DETD(78)

High affinity single-stranded DNA (ssDNA) ligands for thrombin were isolated by SELEX. Two populations of approximately 10.sup.14 ssDNA molecules with either a 30-nucleotide (30N) or 60-nucleotide (60N) variable region and 5' and 3' fixed regions were **synthesized** for the initial selection. Thrombin and DNA were incubated in a buffer containing 50 mM Tris-Cl, pH 7.5, 100 mM NaCl, 1 mM Mg Cl.sub.2 at 37.degree. C. for 5 minutes. The thrombin-bound DNA was partitioned from unbound DNA by nitrocellulose-filter binding. DNA was eluted from the filters by denaturation and phenol/chloroform extraction. A double-stranded DNA product with 3 biotin molecules at the 5' end of the complementary strand was created and amplified by PCR using a 3' complimentary biotinylated primer and sense 5' primer. The double-stranded product was bound to a streptavidin-agrose **matrix** and the nonbiotinylated ss DNA template was isolated by alkaline denaturation. This ss DNA template pool was used for the following round of SELEX.

11. 5,541,311, Jul. 30, 1996, Nucleic acid encoding synthesis-deficient thermostable DNA polymerase; James E. Dahlberg, et al., 536/23.7; 435/252.8, 320.1 [IMAGE AVAILABLE]

US PAT NO: 5,541,311 [IMAGE AVAILABLE] L7: 11 of 20

ABSTRACT:
A means for cleaving a nucleic acid cleavage structure in a site-specific manner is disclosed. A cleaving enzyme having 5' nuclease activity without interfering nucleic acid synthetic ability is employed as the basis of a novel method of detection of specific nucleic acid sequences. Cleaving enzymes are produced through the use of novel DNA sequences which encode novel thermostable polymerases.

SUMMARY:

BSUM(12)

The immobilization of target nucleic acids to solid surfaces such as nylon or nitrocellulose is a common practice in molecular biology. Immobilization formats eliminate the reassociation problem that can occur between complementary strands of target molecules, but not the problems associated with secondary structure effects. However, these mixed phase formats (i.e., Southern hybridization or dot blot hybridization) require time consuming fixation procedures. The hybridization reaction itself is kinetically much slower than a solution phase hybridization reaction. Together, the fixation and hybridization procedures require a minimum of several hours to several days to perform. Additionally, the standard immobilization procedures are often inefficient and result in the attachment of many of the target **molecules** to **multiple** portions on the solid surface, rendering them incapable of subsequent hybridization to probe molecules. Overall, these combined effects result in just a few percent of the initial target molecules being bound by probes in a hybridization reaction.

DETDESC:

DETD(78)

The following is an example of the dual capture assay to detect an antigen(s): A sample to be analyzed for a given antigen(s) is provided. This sample may comprise a mixture of cells; for example, cells infected with viruses display virally-encoded antigens on their surface. If the antigen(s) to be detected are present in solution, they are first attached to a **solid** **support** such as the wall of a microtiter dish or to a bead using conventional methodologies. The sample is then mixed with 1) the **synthetic** domain of a thermostable DNA polymerase conjugated to an antibody which recognizes either a first antigen or a first epitope on an antigen, and 2) the 5' nuclease domain of a thermostable DNA polymerase conjugated to a second antibody which recognizes either a second, distinct antigen or a second epitope on the same antigen as recognized by the antibody conjugated to the **synthetic** domain. Following an appropriate period to allow the interaction of the antibodies with their cognate antigens (conditions will vary depending upon the antibodies used; appropriate conditions are well known in the art), the sample is then washed to remove unbound antibody-enzyme domain complexes. dATP, dTTP and a small amount of poly d(A-T) is then added to the washed sample and the sample is incubated at elevated temperatures (generally in the range of 60.degree.-80.degree. C. and more preferably, 70.degree.-75.degree. C.) to permit the thermostable **synthetic** and 5' nuclease domains to function. If the sample contains the antigen(s) recognized by both separately conjugated domains of the polymerase, then an exponential increase in poly d(A-T) production occurs. If only the antibody conjugated to the **synthetic** domain of the polymerase is present in the sample such that no 5' nuclease domain is present in the washed sample, then only an arithmetic increase in poly d(A-T) is possible. The reaction conditions may be controlled in such a way so that an arithmetic increase in poly d(A-T) is below the threshold of detection. This may be accomplished by controlling the length of time the reaction is allowed to proceed or by adding so little poly d(A-T) to act as template that in the absence of nuclease activity to generate new poly d(A-T) primers very little poly d(A-T) is **synthesized**.

DETDESC:

DETD(81)

The production of poly d(A-T) may be detected in many ways including: 1) use of a radioactive **label** on either the dATP or dTTP supplied for the **synthesis** of the poly d(A-T), followed by size separation of the reaction products and autoradiography; 2) use of a fluorescent probe on the dATP and a biotinylated probe on the dTTP supplied for the **synthesis** of the poly d(A-T), followed by passage of the reaction products over an avidin bead, such as magnetic beads conjugated to avidin; the presence of the florescent probe on the avidin-containing bead indicates that poly d(A-T) has been formed as the fluorescent probe will stick to the avidin bead only if the fluorescenated dATP is incorporated into a covalent linkage with the biotinylated dTTP; and 3) changes fluorescence polarization indicating an increase in size. Other means of detecting the presence of poly d(A-T) include the use of intercalating fluorescence indicators to **monitor** the increase in duplex DNA formation.

DETDESC:

DETD(107)

To determine whether other 5' nucleases in other DNAPs would be suitable for the present invention, an **array** of enzymes, several of which were reported in the literature to be free of apparent 5' nuclease activity, were examined. The ability of these other enzymes to cleave nucleic acids in a structure-specific manner was tested using the hairpin substrate shown in FIG. 6 under conditions reported to be optimal for **synthesis** by each enzyme.

12. 5,240,912, Aug. 31, 1993, Transforming growth factor (TGF) peptides; George J. Todaro, 514/12, 13, 14, 15, 16; 530/324, 325, 326, 327, 328 [IMAGE AVAILABLE]

US PAT NO: 5,240,912 [IMAGE AVAILABLE] L7: 12 of 20

ABSTRACT:
Novel biologically active polypeptides, including a new class of transforming growth factor (TGF) polypeptides, which exhibit cell growth promoting properties are disclosed, as well as a process for isolating the TGF polypeptides from both human and murine cell lines in homogeneous form. Also disclosed are antigenic oligopeptides derived from the TGF polypeptides and antibodies raised therefrom which have application in the detection and treatment of malignancies and oligopeptides have the ability to bind with cellular growth factor receptors and thus to interfere with transformation of certain cell lines into a cancerous state. Compositions and methods based on the disclosed peptides for detection and treatment of cancer and other proliferative diseases and for cell or tissue growth associated treatment, e.g., wound healing, ulcer therapy and bone loss are also described.

DETDESC:

DETD(37)

The polypeptides and oligopeptides according to the invention as defined by the structural formulas (formulas I through IV) and peptide sequences given above can be prepared by **synthetic** techniques, techniques whereby the peptide is isolated from a naturally occurring source, e.g., cell lines and body fluids, and by techniques employing hybrid DNA technology. For those polypeptides and oligopeptides of the invention containing up to about 50 amino acid residues, conventional **solid** phase peptide **synthesis** is suitably employed. In this general **synthetic** procedure for making peptides, which is described, for example, in U.S. Pat. No. 4,341,761 to Ganfield et al., employs known side-chain protecting groups and conventional polystyrene resins supports--e.g., chloromethylated resins, hydroxymethyl resins or benzhydrylamine resins--to affect the amino acid coupling. For polypeptides containing in excess of about 50 amino acid residues, the process according to the invention for isolating homogeneous TGFs from natural sources (which is described in detail below) can be suitably employed to obtain pure forms of the desired peptide. In this regard, particularly suitable sources of the TGF polypeptides according to the invention include serum-free medium conditioned by retrovirus-transformed Fischer rat embryo fibroblasts, in particular fibroblasts transformed with Snyder-Theilen feline sarcoma virus, Moloney murine sarcoma virus-transformed mouse 3T3 cells and human metastatic melanoma cell lines A2058 and A375. Sources and methods for suitable murine cell lines are described in DeLarco et al. (1980) J. Biol. Chem. 255. pp. 3685-3690 and Ozanne et al. (1980) J. Cell. Physiol. 105, pp. 163-180. Sources and methods for human cell lines are similarly described in Todaro et al. (1980) Proc. Natl. Acad. Sci. USA 77, pp. 5258-5262 and Giard et al. (1973) J. Natl. Cancer Inst. 51, pp. 1417-1423. The isolation process of the invention described below can also be used to obtain TGF polypeptides according to the invention from various body fluids such as urine, serum, plasma, whole blood or cerebrospinal fluid of human or murine subjects carrying malignancies or transformed cells which produce TGF polypeptides. In this regard, a suitable source of TGF polypeptides according to the invention is the urine or other body fluids of mice which have been inoculated with tumor cells (human melanoma or transformed rat) known to produce TGF polypeptides. In all cases the identification and purity of the TGF polypeptide can be monitored by a radioreceptor assay based on receptor cross-reactivity with EGF (see experimental examples below). In techniques utilizing recombinant or hybrid DNA technology, the oligopeptides according to the invention or segments of the polypeptides according to the invention containing up to, for example, 20 amino acids can be used to deduce the codon sequence for single stranded nucleotide (DNA) probes. These nucleotide probes can then be **synthesized** using known **synthetic** techniques and used as a probe to obtain messenger RNA (mRNA) coding for growth factor-type polypeptides in both normal and transformed cells or body fluids containing said peptides. Once messenger RNA is obtained, conventional techniques can be used for reverse transcribing of the mRNA to complementary DNA (cDNA) and subsequent cloning of the cDNA in a suitable vector to obtain expression of the desired polypeptide.

DETDESC:

DETD(122)
was **synthesized** using the **solid**-phase technique of Ohgak et al. (1983) Journal of Immunol. Meth. 57, pp. 171-184. This oligopeptide was then coupled to keyhole limpet hemocyanin in accordance with the

procedure of Baron et al. (1982) Cell 28, pp. 395-404, and used to immunize rabbits (Baron et al. (1982) Cell 28, pp. 395-404) and sheep (Lerner (1982) Nature 299, pp. 592-596). Antisera were assayed against peptide by a peroxidase-linked immunoassay (Kirkegaard and Perry Laboratories, Gaithersberg, Md.) and against homogeneous rat TGF (purified according to Example II above), by immunoprecipitation (Bister et al. (1980) J. Virol. 36, pp. 617-621) and Western blotting techniques (Burnett (1981) Analyt. Biochem. 112, pp. 195-203). Binding of .sup.125 I-labeled rat TGF and mouse EGF (Bethesda Research Labs, Bethesda, Md.) to A431 cells grown in 96-well microtiter plates was as described in Pross et al. (1977) Proc. Natl. Acad. Sci. USA 74, pp. 3918-3921.

DETDESC:

DETD(130)

The chemical **synthesis** of rat TGF, having the chemical formula given in Example II, was performed manually by the stepwise **solid**-phase approach according to the general principles described by Merrifield (1963) J. Amer. Chem. Soc. 85, pp. 2149-2156. The differential acid-labile protecting group strategy was adopted for this **synthesis** with the conventional combination of tertbutyloxycarbonyl for N-amino terminus and benzyl alcohol derivatives for the side chains. A more acid stable benzyl ester linkage that anchored protected amino acids to the polymeric **support** was used to minimize loss of peptides during the repetitive acid treatments (Mitchell et al. (1976) J. Amer. Chem. Soc. 92, pp. 7357-7362). Complete deprotection and removal of peptide from the resin was by the low-high HF method of Tam et al.(1983) Tetrahedron Lett. 23, pp. 4435-4438, which differed from the conventional HF deprotection method and removed benzyl protecting groups by the S.sub.N 2 mechanism in dilute HF solution to minimize serious side reactions due to carbocations generated in the conventional S.sub.N 1 deprotection method. Furthermore, it is also designed to reduce many cysteinyl side reactions that often hamper the **synthesis** of proteins containing **multiple** disulfide linkages.

DETDESC:

DETD(132)

Under reducing or nonreducing conditions, the purified **synthetic** rTGF was found to give a single band with an apparent molecular weight of 7000 on SDS-PAGE electrophoresis. Amino acid analysis by 6N HCl and enzymatic hydrolysis provided the expected theoretical molar ratio of the proposed sequence. No free thiol was detected by Ellman's method of sulhydryl determination on **synthetic** rTGF, but upon thiolytic reduction, the expected theoretical value of six cysteines was obtained. These findings **support** the conclusion that **synthetic** rTGF is a single chain polypeptide containing six cysteines in disulfide linkages, which is in agreement with the expected chemical properties of the natural rTGF. Additionally, **synthetic** rTGF coeluted with the natural rTGF as a single symmetrical peak in C.sub.18 reverse phase HPLC.

DETDESC:

DETD(193)

Peptides, corresponding to amino acids from portions of rTGF amino acid sequence, described above in Example II, were **synthesized** commercially (Peninsula Labs) by the standard **solid** phase technique of Ohgak et al. (1983) Journal of Immunol. Meth. 57, pp. 171-184. If necessary, peptides were purified by reverse phase high performance liquid chromatography (HPLC) prior to use.

DETDESC:

DETD(251)

Synthetic rat TGF was prepared as described above in Example V. Purity of the protein was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, amino acid analysis, and reverse phase high-performance liquid chromatography. The biological activity of TGF preparations and **synthetic** TGF was **monitored** by means of an EGF radioreceptor assay. (Todaro et al. (1976) Nature 264, 26.) Bone resorption was assessed by measuring the release of .sup.45 Ca from previously **labeled** fetal rat long bones. Pregnant rats at the 18th day of gestation were injected with 200 .mu.Ci of .sup.45 Ca. (Raisz (1975) J. Clin. Invest. 44, 103.) The mothers were killed on the 19th day of gestation, and the fetuses were removed. The mineralized shafts of the radii and ulnae were dissected free of surrounding tissue and cartilage and placed in organ culture. The bones were incubated in BGJb medium (Irvine Scientific) for 24 hours at 37.degree. C. in a humidified atmosphere of 5 percent of CO.sub.2 and 95 percent air to allow for the exchange of loosely complexed .sup.45 Ca. The bones were then cultured for 48 to 120 hours in BGJb medium supplemented with 5 percent fetal calf serum (KC Biologicals) containing control or test substances. Bone-resorbing activity was measured as the percentage of total .sup.45 Ca released into the medium and was expressed as a treated-to-control ratio. Statistical significance was determined with Student's t test for unpaired data.

13. 5,221,518, Jun. 22, 1993, DNA sequencing apparatus; Randell L. Mills, 422/62, 67, 82.05; 435/284.1, 287.2; 436/89 [IMAGE AVAILABLE]

US PAT NO: 5,221,518 [IMAGE AVAILABLE] L7: 13 of 20

ABSTRACT:
The DNA sequencing apparatus contains the following components. A first reaction vessel contains a reaction chamber into and out of which can be transferred reagents, reactants and reaction products. A device for separating individual oligonucleotides and polynucleotides on the basis of length, such as an electrophoretic unit, receives reaction products from the first reaction vessel. A second reaction vessel is designed for oxidizing pentose sugars. It comprises a reaction chamber having a device for transferring reactants and reagents into the reaction chamber and gaseous by-products of a reaction out of the reaction chamber. Separated oligonucleotides and polynucleotides can be selectably transferred from the separating device alternatively into the first or the second reaction vessel. The device also includes a second transfer device for transferring the gaseous by-products out of the second reaction vessel and a collection chamber for collecting the gaseous by-products. The collection chamber is in communication with the second transfer device. Finally, the apparatus contains an analyzer for analyzing the relative abundance of the components of the gaseous by-product by mass such as a mass spectrometer, the analyzer being in communication with a transfer device for transferring gaseous by-products from the collection chamber to the analyzer.

SUMMARY:

BSUM(18)

In the preferred embodiment of the invention, the polynucleotides are RNA/DNA hybrid molecules generated from the DNA to be sequenced. To form these hybrids, DNA to be sequenced is broken into fragments and each fragment used as a template to form one or more RNA transcript(s). The RNA transcript(s) is then extended on the original intact DNA template with deoxyribonucleotides to form the DNA portion of the hybrid(s). The extension is terminated randomly by addition of dideoxynucleotides to the polymerase reaction. This yields RNA/DNA hybrid **molecules** which are "**random**" in length at the 3' end. The molecules can then be randomized at the 5' (RNA) end preferably by using an RNA exonuclease which under appropriate conditions, degrades the 5' RNA portion. The result of this procedure is a family of polynucleotides having the characteristic set forth above. The "axis" referred to above is the dividing line between the RNA and DNA and it immediately follows the 3' most ribonucleotide of all the hybrid molecules.

DETDESC:

DETD(113)

In the second procedure, which is the preferred method, RNA copies are made without use of a primer. If ribonucleotides are added to the reaction mixture in high concentration in the absence of sigma factor, which controls initiation of RNA polymerization only from special DNA sequences called promoters, then RNA polymerase initiates RNA **synthesis** at **random** points along the DNA template and a free end stimulates initiation at that site. The procedure is carried out in substantially the same way as for primed RNA synthesis except that no primers are added and a concentration of about 2 millimolar ribonucleotides is used.

DETDESC:

DETD(157)

DNA or RNA bands collected as fractions can be **monitored** spectrophotometrically at wavelengths of 260 and 280 nm or by ethidium bromide fluorescent quantitation. See Maniatis, pp. 468-469; 163. The preferred method of ethidium bromide fluorescent quantitation is to use ethidium bromide in the gel or in the anode well at a concentration of about 0.5 ug/ml. See Maniatis, p. 163. Also, it is not necessary to remove ethidium bromide from RNA or DNA to perform primed **synthesis** of Step III. (See Methods of Enzymology; vol. 65, p. 565 (1980)). If the mass **labeled** nucleotides contain in addition a radiolabel, then the bands can be **monitored** by a scintilation counter.

DETDESC:

DETD(237)

In reference to Methods I, two procedures of producing RNA copies of the single stranded restriction fragments are described. See pages 53-54. Procedure A involves using primed RNA **synthesis**; Procedure B involves using RNA polymerase under conditions that allow the enzyme to initiate RNA polymerization randomly on a DNA template with high activity. Both methods may give rise to subsets that have a common 5' end. In this case the simple strategy described below is used to solve for the sequence from the data. But, the replication products may not all have a common 5' end; therefore, the data must be treated as if subsets are made from the loss of nucleotides randomly from the 3' and 5' end of the largest polynucleotide and the **matrix** method of analysis must be used as described in this discussion.

DETDESC:

DETD(261)

For the preferred method, since the 3' **random** hybrid **molecules** are 5' randomized by a procedure which only removes RNA, the most 3' junction of RNA and DNA relative to the complement of the parent represents the "axis". Furthermore, if one RNA copy is isolated when following the procedure described in Methods I, then each of the polynucleotides generated from subsequent reactions and scanned, yields the solution of one nucleotide. Thus, the ratio of the number of "unknowns" solved to the total number of polynucleotides generated is one. If more than one RNA copy is isolated, then the ratio is x/xR=1/R, where R is the number of RNA copies isolated.

14. 5,185,147, Feb. 9, 1993, Short polypeptide sequences useful in the production and detection of antibodies against human immunodeficiency virus; Lawrence D. Papsidero, 424/188.1, 208.1; 514/13, 14, 15, 16, 17; 530/326, 327, 328, 329, 387.2, 387.9, 388.35, 402, 403, 826; 930/221 [IMAGE AVAILABLE]

US PAT NO: 5,185,147 [IMAGE AVAILABLE] L7: 14 of 20

ABSTRACT:
Polypeptides in the size range 6-11 amino acids from discrete regions of the human immunodeficiency virus p17 protein are immunogenic and form the basis for diagnosis and therapy of HIV-related disease.

DRAWING DESC:

DRWD(10)

FIG. 5. Epitope mapping of monoclonal antibodies to p17 using epitope scanning (Geysen technique). A series of sequential, overlapping hexapeptides were **synthesized** in situ on **solid** phase pins, as described in Materials and Methods. The peptide series corresponds to the entire HIV p17 reading frame, beginning at the ATG (met) start codon. The peptides were probed for immunoreactivity against monoclonal anti-p17 antibodies (clones 32/5.8.42 and 32/1.24.89) at 10 ug/ml. The reactions were developed using biotin-labeled goat antibodies to murine IgG followed by streptavidin-peroxidase and then substrate. Results are expressed as optical density (ordinate) versus peptide number (abscissa).

DETDESC:

DETD(27)

Polypeptide multimers may be prepared by bonding together the **synthesized** polypeptide monomers in a head-to-tail manner using the aforementioned **solid** phase method; i.e., one complete polypeptide sequence can be **synthesized** on the resin, followed by one or more of the same or different polypeptide sequences, with the entire multimeric unit thereafter being cleaved from the resin and used as described herein. Such head-to-tail polypeptide multimers preferably contain about 2 to 4 polypeptide repeating units.

DETDESC:

DETD(41)

The polypeptides of this invention are chemically **synthesized** by **solid**-phase methods as previously described (18, 30) [See also U.S. Pat. No. 4,316,891, issued to Guillemin et al.] The **solid** phase method of polypeptide **synthesis** is practiced utilizing a Beckman Model 990B Polypeptide **Synthesizer**, available commercially from Beckman Instrument Co., Berkeley, Calif., or an equivalent instrument.

DETDESC:

DETD(43)

In preparing a **synthetic** polypeptide of this invention by the above **solid** phase method, the amino acid residues are linked to a resin (**solid** phase) through an ester linkage from the carboxy-terminal residue. When the polypeptide is to be linked to a carrier via a Cys residue or polymerized via terminal Cys residues, it is convenient to utilize that Cys residue as the carboxy-terminal residue that is ester-bonded to the resin.

DETDESC:

DETD(50)

It is also to be noted that a **synthetic** multimer of this invention can be prepared by the **solid** phase, **synthesis** of a plurality of the polypeptides of this invention linked together end-to-end (head-to-tail) by an amide bond between the carboxyl-terminal residue of one polypeptide and the amino-terminal residue of a second polypeptide "peptide bond". Such **synthetic** multimers are preferably **synthesized** as a single long polypeptide multimer, but can also be prepared as individual polypeptides that are linked together subsequent to their individual **synthesis**, using a carbodiimide reagent such as 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride in water. The total number of amino acid residues contained in a multimer prepared as a single polypeptide chain is preferably less than about 50. A **synthetic** head-to-tail multimer more preferably contains two to about four blocks of linked, **synthetic**, **random** copolymer polypeptides of this invention, and a total of less than about 40 amino acid residues.

DETDESC:

DETD(54)

The polymer so prepared contains a plurality of the **synthetic**, **random** copolymer polypeptide repeating units that are bonded together by oxidizing cysteine (cystine) residues. Such polymers typically contain their polypeptide repeating units bonded together in a head-to-tail manner as well as in head-to-head and tail-to-tail manners; i.e., the amino-termini of two polypeptide repeating units may be bonded together through a a single cystine residue as may two carboxyl-termini since the linking groups at both polypeptide termini are identical.

DETDESC:

DETD(101)

Solid-phase **synthetic** peptides were examined for their immunoreactivity using enzyme immunoassay (EIA). Polyethylene pins, with peptides on their surface (see below), were counter-coated in EIA buffer (PBS/1% ovalbumin/1% bovine serum albumin/0.1% Tween-20) for 18 hours at 4 degrees C. After washing with PBS-T (4.times.10 min), the pins were incubated in microplates containing MAb or control antibody, each at 10 ug/ml, for 18 hours at 4.degree. C. After washing as above, incubation was allowed to proceed for 1 hour in enzyme-conjugated antiglobulin (anti-murine IgG: peroxidase; Jackson Labs). The washed pins were next immersed into wells containing ABTS substrate solution (azino-bis-3-ethylbenzthiazoline-6-sulfonic acid, 0.5 mg/ml in pH 4.0 citrate buffer/0.03% hydrogen peroxide). Reactions were stopped after 30 minutes by removing the pins and absorbance measurements were taken at 450 nm using a microplate reader.

DETDESC:

DETD(102)

Competitive inhibition experiments were performed with soluble, **synthetic** peptides dissolved in PBS-T. **Solid**-phase target antigen represented 96-well microplates which were coated with HIV (5 ug/ml) for 18 hours at 4 degrees C. For competition analysis, various concentrations of **synthetic** peptides were allowed to react within HIV-coated microwells in the presence of biotin-**labeled** MAbs for 90 minutes at 37 degrees C. Biotin derivitization was performed using N-hydroxysuccinimide-d-biotin (Calbiochem, La Jolla, Calif.) (15). The concentration of biotin-MAb chosen corresponded to approximately 40% of maximal binding activity. After aspiration of the probe:inhibitor mixture, the wells were washed five times with PBS-T and streptavidin-peroxidase (Jackson Labs) was added for a further 30 minutes. Thereafter, washed wells received ABTS substrate solution and absorbance was **monitored** as above. Specific inhibition was calculated according to the formula:

DETDESC:

DETD(105)

Epitope scanning. The strategy employed consisted of the construction of sequential, overlapping hexapeptides which completely spanned the entire HIV p17 amino acid sequence (16). Since the anti-p17 MAbs under study strongly reacted with the prototype HTLV-IIIB strain of HIV, its published sequences were used to construct peptide homologs (17). Peptides were **synthesized** in situ on plastic pins which conform in configuration to a standard 96-well microplate, using reagents and a kit (Epitope Mapping Kit) provided by Cambridge Research Biochemicals, Inc., Valley Stream, N.Y. After step-wise, **solid**-phase **syntheses**, the peptides were de-protected (20%) piperidine in dimethyl formamide), washed and air-dried. Included in the experiment was the use of concurrently-**synthesized** peptide controls with known reactivity versus available antisera. These peptides represented the sequences Pro Leu Ala Gln and Gly Leu Ala Gln. One of these peptides (Pro Leu Ala Gln) is known to react with antibody to sperm whale myoglobin, while the other is non-reactive but similar in structure. These EIA-testable peptides were included in each assay run.

DETDESC:

DETD(114)

To confirm the data obtained from epitope scanning experiments, soluble peptides were **synthesized** which corresponded to the amino-terminal, MAb 32/5.8.42-binding site (epitope "A"; residues 12-19); the MAb 32/1.24.89-binding site (epitope "B"; residues 17-22); and to a region containing both binding sites (epitope "A/B"; residues 12-22): these **synthetic** peptides were termed SP-17-A, SP-17-B and SP-17-A/B, respectively. At the experimental level, each soluble peptide was allowed to compete with **solid**-phase HIV for the binding of both MAbs. As shown in FIG. 6, SP-17-A effectively inhibited the binding activity of MAb 32/5.8.42, exhibiting an ID.sub.50 dose of approximately 1 ug/ml. This peptide was immunologically specific, inasmuch as no effect was noted on the reactivity of MAb 32/1.24.89. SP-17-B, corresponding to the binding site of MAb 32/1.24.89, was capable of inhibiting homologous antibody but only at very high concentrations (ID.sub.50 of approximately 3.2.times.10.sup.3 ug/ml), indicating a low affinity interaction. However, the inhibition was immunologically specific. Further studies was a **synthetic** peptide which contained both MAb binding sites. This peptide, SP-17-A/B was a strong inhibitor of each anti-p17 antibody (FIG. 6). The ID.sub.50 dose versus MAb 32/5.8.42 was similar to that observed with SP-17-A (0.4 versus 1.06 ug/ml). In distinction, SP-7-A/B was almost 500 times more effective than SP-17-B with respect to its capacity to compete MAb 32/1.24.89 (ID.sub.50 dose 6.03 ug/ml versus 3.2.times.10.sup.3 ug/ml). Of the three **synthetic** peptides studied, none demonstrated any detectable inhibition of an irrelevant MAb (anti-HIV p24, clone 32/5.17.76), at dose ranges of up to 10.sup.3 ug/ml.

15. 5,137,816, Aug. 11, 1992, Rhizobial diagnostic probes and rhizobium trifolii nifH promoters; Barry G. Rolfe, et al., 435/172.3, 252.2, 252.3, 320.1, 878; 536/23.6, 23.71; 935/41, 72 [IMAGE AVAILABLE]

US PAT NO: 5,137,816 [IMAGE AVAILABLE] L7: 15 of 20

ABSTRACT:
This invention provides useful promoters from the R. trifolii nifH gene for the construction of recombinant molecules to regulate foreign genes for expression under desired conditions. In particular, the promoters act to control expression of the foreign genes within root nodules formed by rhizobial bacterial strains in symbiotic combination with host plants. A rhizobium diagnostic segment (RDS) is also provided comprising a DNA segment found at more than one location in rhizobia, the RDS being species-specific, and detectable by DNA hybridization under stringent conditions. A recombinant plasmid comprising a RDS and a bacterial strain containing the plasmid are provided. Methods are provided for identifying species and strains of field isolates of Rhizobium, using RDS's. One RDS exemplified comprises 5' sequences from the R. trifolii nifH gene.

SUMMARY:

BSUM(5)

Prokaryotes can use a wide variety of nitrogen compounds as sole sources of cellular nitrogen. This variety includes ammonia, dinitrogen and nitrate among the inorganic compounds, and proline, arginine and glutamine among complex organic compounds. Each species can utilize a

different **array** of nitrogen compounds. Glutamine, glutamate and aspartate are the key nitrogen compounds in intermediary metabolism. The latter two are the starting compounds of many pathways of amino acid biosynthesis and serve as amino group donors in many reactions. In all other cases the amino group is donated by glutamine. The major enzyme required for the assimilation of ammonia is glutamine **synthetase**, which catalyses the reaction:

DETDESC:

DETD(126)

Recombinant plasmids containing inserts of the gene encoding the toxic crystal protein of B. thuringiensis are obtained using the techniques described (Wong, H. C. et al. (1983) J. Biol. Chem. 258:1960-1967). The recombinant plasmid pES1 (ATCC Number 31995) consisting of the plasmid vector pBR322 and DNA homologous to the 30, 32 and 37 megadalton plasmids, as well as DNA homologous to linearized forms of the very large plasmids of B. thuringiensis is partially cleaved with EcoRI to give linear molecules. These partial cleavage products are further restricted by the enzyme AvaI. The digestion conditions are as recommended by the manufacturer. A probe for the toxic crystal protein gene is isolated and radioactively **labelled** as previously described (Wong, H. C. et al. (1983) see supra). The restriction fragments are separated by agarose gel electrophoresis and the **labelled** probe is found to hybridize to one fragment of approximately 15 kilobases (kb). This fragment includes the EcoRI fragments D and F (Wong et al., supra). The 15 kilobase fragment is then cloned into M13mp8 or M13mp9 according to standard procedure (Messing, J. and J. Vieira (1982) Gene 19:269-276) and transformed into E. coli JM103. The single stranded DNA from the extruded phage particles is purified and replicated in-vitro by use of a **synthetic** primer (5'-TGTTATCCATGGGTTACCTCC-3'). (The general method of site specific mutagenesis is described in Zoller, M. J. and M. Smith (1982) Nucleic Acids Research 10:6487-6500.) The resulting double-stranded recombinant plasmid is then transformed back into E. coli JM103 and amplified. The amplified double-stranded plasmid DNA is purified from the E. coli JM103 cells and cleaved with the restriction endonuclease NcoI and AvaI. NcoI cleaves at the site of the **synthetic** primer (which is the initiation site of the toxic crystal protein gene) and AvaI cleaves at a site which is downstream from the 3'-end of the toxic crystal protein gene. The overhangs are then filled in to blunt ends (Maniatis, T. et al. (1975) supra). Finally the pSS204 recombinant plasmid which is derived from pSUP204 is cleaved with EcoRI and the overhangs filled in to blunt ends. HindIII linkers are then added to both the B. thuringiensis toxic crystal protein gene fragment and to the pSS204 recombinant. Following the HindIII digestion of both components, the toxic crystal protein gene and the pSS204 recombinant plasmid are ligated together to give a pSS204-B. thuringiensis toxic crystal protein gene cointegrate. The mixture is transformed into a suitable E. coli host, e.g., K802, SM10 or RR1. Plasmids are isolated from individual colonies and the orientation determined by restriction mapping. A colony containing a plasmid with the correct orientation is then conjugated to a rhizobial strain and the plasmid is transferred as already described. The production of mRNA and/or the toxic crystal protein is **monitored** as already described (Wong et al., supra).

DETDESC:

DETD(157)

Radioactive probes were prepared by primed **synthesis** using **random** 8-12 nucleotide long oligonucleotide primers made from calf thymus DNA. Template DNA (100-200 ng purified restriction fragment, or 1-2 .mu.g linear plasmid DNA) and 100 .mu.g primer were mixed in 20 .mu.l H.sub.2 O, denatured by boiling for 2 minutes and quick cooled on ice. Synthesis was initiated by the addition of 50 mM Tris-HCl pH 8.0, 20 mM KCl, 7 mM MgCl.sub.2, 10 mM .beta.-mercaptoethanol, 600 .mu.M dGTP, 600 .mu.M dTTP, 600 .mu.M dATP, 0.3 .mu.M .alpha..sup.32 P-dCTP (3000 Ci/mmole, Amersham) and 5 units E. coli DNA polymerase I (Klenow fragment). This mix was incubated at 37.degree. C. for 30 minutes. For probes with higher specific activities the 600 .mu.M dATP was replaced with 0.3 .mu.M .beta..sup.32 P-dATP (3000 Ci/mmole). The reaction was stopped with 25 mM EDTA and extracted with phenol and chloroform.

16. 5,089,406, Feb. 18, 1992, Method of producing a gene cassette coding for polypeptides with repeating amino acid sequences; Jon I. Williams, et al., 435/172.3, 69.1; 530/353, 356; 935/10 [IMAGE AVAILABLE]

US PAT NO: 5,089,406 [IMAGE AVAILABLE] L7: 16 of 20

ABSTRACT:
This invention relates to processes for the microbial production of peptide oligomers, to polypeptide products resulting from application of any of these processes, and to microbes for use in such production. Another aspect of this invention relates to processes for genetically engineering such microbes and to plasmid vectors for use in such engineering.

DETDESC:

DETD(2)

One aspect of this invention relates to a process for forming double-stranded DNA fragments which code for a desired repeating amino acid sequence with linker DNA ends which may be inserted into a suitable plasmid vector. As part of the first step of the process at least two **synthetic** oligodeoxynucleotides which can function as coding or anticoding strands for a desired amino acid sequence are prepared. Oligodeoxynucleotides are polymeric DNA sequences which are linear chains of deoxynucleotides covalently linked through a phosphodiester bond between the C5' and C3' atoms of adjacent deoxyribose sugar moieties. The **synthetic** method for preparing such oligodeoxynucleotides sequences may vary widely. For example, they can each be chemically **synthesized** by any one of several available solution or **solid** phase techniques. See M.H. Caruthers et al., Genetic Engineering, Volume 4 (J. Setlow and A. Hollaender, eds.; Plenum Press, 1982) for a review of the preferred **solid** phase **synthesis** technology based on phosphoramidite chemistries as originally disclosed in S.L. Beaucage and M. Caruthers, Tetrahedron Letters 22, 1859-1862 (1981).

DETDESC:

DETD(8)

The choice of nucleotide sequence in the synthetic oligodeoxynucleotides is governed by the order of amino acids in the basic repeating unit for which directly repeating oligomers are desired in product polypeptides. One or more of the synthetic oligodeoxynucleotides can then be selected to code for the desired basic repeating peptide unit or a circularly permuted version of this coding sequence. Coding sequences are chosen on the basis of the genetic code and preferred codon usage in the host microorganism in which the synthetic gene described in this invention is to be expressed. More than one coding sequence may be chosen in situations where codon preference is unknown or ambiguous for optimum codon usage in the chosen host microorganism. The length of the selected or prepared oligodeoxynucleotides may vary widely. The minimum length of the oligodeoxynucleotide for use in the process of this invention is a number of covalently joined nucleotides which is equal to three times the number of amino acids in the basic repeating peptide unit. The maximum length is not critical and the employment of **synthetic** deoxynucleotides with integral **multiples** of this number of bases is also acceptable and is preferred if the number of amino acids in the basic repeating peptide unit is less than about 4.

DETDESC:

DETD(16)

Following treatment with a DNA polymerase, the **synthetic** double-stranded DNA fragments prepared in certain embodiments of the invention are fractionated to isolate only those fragments of greater than some minimum size for use in subsequent process steps. This purification procedure may also be necessary for any natural genes, gene fragments or DNA copies of messenger RNAs for specific genes or gene fragments which are of utility in certain embodiments of the process of this invention as described below. The method of purification can be chosen from a variety of biochemical techniques including size exclusion chromatography, ion exchange chromatography and affinity chromatography. The current preferred method is size exclusion chromatography over a suitable separation **matrix**; many such matrices are commercially available.

DETDESC:

DETD(38)

The following complementary and overlapping oligodeoxynucleotides were prepared using **solid** phase phosphoramidite chemistry as disclosed in Beaucage and Caruthers, op. cit., on an Applied Biosystems model 380 DNA **synthesizer**: ##STR13## Each oligodeoxynucleotide was isolated from shorter chain-elongation failure products by electrophoresis on and elution from 20% polyacrylamide gels containing 8 M urea. The final product was greater than 95% pure as determined by densitometry of autoradiograms prepared from end-labeled oligodeoxynucleotide products separated by analytical gel electrophoresis. Phosphate was added to the 5' ends of oligodeoxynucleotides A and B in separate reactions that contained 8.6 nmol oligodeoxynucleotide and 20 units T4 polynucleotide kinase in 35-45 ul buffer (66 mM Tris-HCl, oH 7.6, 1 mM spermidine, 10 mM MgCl.sub.2, 15 mM dithiothreitol, 200 ug/ml bovine serum albumin (BSA), and 1 mM [.gamma.-.spsp.32 P]ATP with a specific activity of 0.2 Ci/mmol). These reaction mixtures were incubated for 2 hr at 37.degree. C., then they were combined and were incubated at 14.degree. C. overnight. During this time, oligodeoxy nucleotides A and B were annealing, presumably to form 17 base pair heteroduplexes with one base pair overhanging 3' ends or 10 base pair heteroduplexes with 8 base pair overhanging 5' ends. T4 DNA ligase (40 units) was added and incubation was continued at 14.degree. C. for three days to polymerize the annealed oligodeoxynucleotides into long repetitive heteroduplex DNA coding for multiple repeats of the tripeptide (Gly-Pro-Pro). These **synthetic** genes were dialyzed against TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) to remove unincorporated oligodeoxynucleotides and buffer components. The ends of the **synthetic** genes were then blunt-ended by using three units of the Klenow fragment of E. coli DNA polymerase I in a reaction (50 ul total volume) containing the following: 600 uM each of dCTP, dGTP, dATP and TTP; 50 mM Tris-HCl, oH 7.8; 9mM MgCl.sub.2 ; 10 mM 2-mercaptoethanol; and 50 ug/ml BSA. This reaction mixture was incubated at 14.degree. C. for 30 minutes, then Na3EDTA was added to 10 mM and 150 ul of TE buffer was also added. The **synthetic** genes were purified on a DE-52 column, then ethanol precipitated. These **synthetic** genes were combined with the excluded fraction of another batch of **synthetic** genes prepared in substantially like manner that had previously been passed over a Sepharose 6B (Pharmacia) column. The combined **synthetic** genes were size fractionated on a Sepharose 4B (Pharmacia) column. The size distribution of **synthetic** genes was determined by electrophoresis on a 5% polyacrylamide gel.

DETDESC:

DETD(49)

Direct sequencing of the 5' junctions of the **synthetic** gene insert in several supercoiled plasmid DNAs bearing a **synthetic** collagen analogue gene without DNA linkers was conducted as disclosed in R. J. Zagursky et al., Gene Analytical Techniques 2: 289-94 (1985). The 5' and 3' gene orientations as used here respectively refer to the proximal and distal junctions relative to the lambda PL promoter located in pJL6. The following oligodeoxynucleotide was prepared by **solid** phase automated

synthesis for priming DNA sequencing reactions based on the Sanger dideoxynucleotide sequencing method as adapted by Zagursky et al.: ##STR14## Primed **synthesis** reactions using this oligodeoxynucleotide allow sequencing into any gene inserted at the Cla.sub.I site of pJL6 and in a direction reading toward the HindIII site of oJL6. On the basis of proper reading frame and correct coding information at both the 5' and 3' junctions, one of these plasmids was designated pACI and investigated further.

DETDESC:

DETD(54)

About 20 ug of each RNA sample was prepared for gel electrophoresis as disclosed in T. Maniatis et al., Molecular Cloning (Cold Soring Harbor, 1982), pp. 202-203. The RNA samples were electrophoresed on a 1.0% agarose-formaldehyde gel at 30 V. overnight. The next morning, the gel was stained with acridine orange to visualize the RNA and processed for Northern hybridization analysis according to the procedure disclosed by Barinaga et al. in Transfer of RNA to **Solid** Supports (Schleicher and Schuell). The agarose-formaldehyde gel was blotted onto DBM paper overnight. Northern prehybridization solution was prepared as described by Barinaga et al. The DBM paper with transferred RNA was incubated in 17 ml prehybridization solution at 42.degree. C. overnight. The probe for the Northern blot consisted of oligodeoxynucleotide B of Example 1 radiolabeled with T4 polynucleotide kinase in the presence of [.gamma.-.sup.32 P]ATP. The hybridization solution consisted of 25% formamide, 5X SSPE, 0.05% SDS, 1 nM Na.sub.2 EDTA, IX Denhardt's solution and 750 ug/ml salmon sperm DNA (see T. Maniatis et al., op. cit., for definition of IX SSPE). The probe and hybridization solution were mixed and incubated with the DBM paper containing transferred RNA at 37.degree. C. overnight. The DBM paper was then washed successively in 1 1 4X SSPE, 0.1% SDS for 20 minutes at 55.degree. C., 800 ml minutes at 55.degree. C,,200 ml of 4X SSPE for 1 minute at 55.degree. and 500 ml of 2X SSPE at room temperature for 2 minutes. The blot was subsequently dried and exposed to X-ray film overnight. The autoradiogram resulting from this exposure showed very strong probe hybridization to DC1139A(pACl) RNA for the culture induced at 41.degree. C. Hybridization in all other strains and under other culture conditions including growth of DC1139A(pACl) at 30.degree. C. was minimal. These data demonstrate unambiguously that strong induction of collagen analogue oligodeoxy-nucleotide B-specific messenger RNA **synthesis** from the .lambda.P.sub.L promoter occurred only at the high temperature and only in strain DC1139A(pACl) as expected.

DETDESC:

DETD(68)

The following oligodeoxynucleotides were synthesized as the first step in constructing pAVl using an Applied Biosystems model 380A automated DNA synthesizer: ##STR15## The oligodeoxynucleotide E is completely complementary to a portion of the oligodeoxynucleotide D and produces a DNA fragment having both 5' and 3' overhanging ends. When annealed, oligodeoxynucleotides D and E form a heteroduplex DNA within which are located restriction enzyme recognition sites for both NdeI and HindIII. The most direct method of constructing oAVl from D and E is to digest the **synthetic** heteroduplex with NdeI and HindIII and then ligate the heteroduplex product into pJL6 from which the small DNA fragment produced by an NdeI-HindIII double digest has been excised. During the course of constructing pAVl, it was determined that NdeI restricted the **synthetic** heteroduplex formed by D and E poorly or not at all, necessitating the additional steps described herein. Oligodeoxynucleotides D and F were annealed (270 pmol of each) in 35 ul of 10 mM TE buffer (see Example 1) by allowing the solution to cool slowly from 75.degree. C. to room temperature. A portion of this **synthetic** heteroduplex was radiolabeled by T4 polynucleotide kinase in the presence of [.lambda.-.spsp.32 P]ATP. After completing the radiolabeling, the **synthetic** heteroduplex was purified by chromatography on DE-5.sub.2 cellulose (Whatman) and then precipitated in ethanol. The **labeled** **synthetic** heteroduplex was added to the unlabeled material as a tracer and the combined fractions were further purified on a NENSORB-20 column (DuPont) and then concentrated by evaporation. Another 270 pmol each of oligodeoxynucleotides D and E were added to the concentrated solution and the annealing reaction was repeated by allowing the solution to cool slowly from 98.degree. C. to 4.degree. C. Proper annealing was **monitored** by gel electrophoresis of an aliquot of the reaction mixture in 16% polyacrylamide.

17. 5,071,963, Dec. 10, 1991, Interferon-induced human (2'-5') oligo a synthetase; Michel Revel, et al., 530/387.9; 435/5, 6, 7.1, 7.4, 7.9, 188, 810; 436/86, 501, 504, 800, 804, 813; 530/326, 389.1, 391.3, 806; 536/23.2; 935/110 [IMAGE AVAILABLE]

US PAT NO: 5,071,963 [IMAGE AVAILABLE] L7: 17 of 20

ABSTRACT:

Human DNA encoding enzymes having (2'-5') oligo A synthetase has been sequenced. The amino acid sequences of the enzymes have been deduced. Antigenic peptides have been prepared and have been used to raise antibodies which recognize and immunoprecipitate the 40 kd, 46 kd, 67 kd and 100 kd forms of (2'-5') oligo A synthetase. Methods of monitoring interferon activity in a subject are presented.

PARENT-CASE:

BACKGROUND OF THE INVENTION

This application is a continuation-in-part of U.S. Ser. No. 833,212, filed Feb. 25, 1986, which is a continuation-in-part of U.S. Ser. No. 601,782, filed Apr. 18, 1984, now abandoned the contents of both of which are hereby incorporated by reference.

Throughout this application, various publications are referenced by the name of the author and date of publication within parentheses. Full citations for these references may be found at the end of the specification listed in alphabetical order immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

Many of the biological effects of interferon (IFN) appear to be mediated by the induction of new mRNAs and proteins in cells exposed to IFNs (for review: Revel, 1984; Lebleu and Content, 1982; Baglioni and Nilsen, 1983). Among these IFN-induced proteins, two groups appear particularly important: 1) translation regulatory enzymes (ds RNA dependent protein kinase and (2'-5') oligo A synthetase, (2'-5') oligo A-activated nuclease, 2-phosphodiesterase); and 2) cell surface antigens (HLA-A, B, C, B2-microglobulin, HLA-DR). Other cellular and excreted proteins probably play important roles as well (Weil et al., 1983; Chebath et al., 1983; Wallach et al., 1983). With the exception of the HLA genes (Malissen et al., 1982; Schamboeck et al., 1983), the structure and sometimes the function of the IFN-induced proteins is unknown and so is the mechanism by which IFNs activate specifically these genes. To address these questions, several cDNAs from IFN-induced genes have been recently cloned (Chebath et al., 1983; Merlin et al., 1983; Friedman et al., 1984; Samanta et al., 1984). We have, in particular, studied the cDNA and gene coding for the human (2'-5') oligo A synthetase (OASE), a ds RNA-activated enzyme that converts ATP into ppp(A2'pA)n oligomers (Kerr and Brown, 1978) which in turn bind to and activate the latent RNAse F (Schmidt et al., 1978). The (2'-5') oligo A synthetase is strongly induced in cells by all three types of human IFNs, and its increase is a good marker of IFN activity (Wallach et al., 1982). The enzyme is induced during differentiation of hematopoietic cells, and denotes an autocrine secretion of IFN-beta (Yarden et al., 1984). The enzyme is similarly induced late in the S phase of synchronized embryo fibroblasts (Wells and Mallucci, 1985). The enzyme activity drops when cell growth starts (Etienne-Smekens et al., 1983; Creasey et al., 1983) and appears to be involved in the antigrowth effect of IFN (Kimchi et al., 1981). Deficiency in the (2'-5') oligo A synthetase or in the (2'-5') oligo A-activated RNAse F has also been correlated with partial loss of the antiviral effects of IFNs (Salzberg et al., 1983; Epstein et al., 1981), although this is probably not the only mechanism by which IFN inhibits virus growth (Lebleu and Content, 1982). The (2'-5') oligo A nucleotides have been detected in many eucaryotic cells and even in bacteria (Laurence et al., 1984) and the synthetase is likely to be a wide-spread enzyme. The enzyme has been purified from mouse (Dougherty et al., 1980) and human cells (Yand et al., 1981); Revel et al., 1981); a large and a small form of the enzyme have been observed (Revel et al., 1982; St. Laurent et al., 1983) but their structures were not elucidated.

The (2'-5') oligo A **synthetase**, induced in cells exposed to IFNs (Hovanessian et al., 1977; Zilberstein et al., 1978) has a number of unusual properties. Its main activity is the **synthesis** from ATP of 5'triphosphorylated short oligo A chains (of up to 15 A, with mainly dimers to pentamers), but in contrast to other RNA polymerases, it adds adenylate or one other nucleotide specifically to the 2'OH of adenylate in oligo A (Kerr and Brown, 1978; Samanta et al., 1980), or to other (oligo) nucleotides with a free 2'OH adenylate such as NAD Ball, 1980) or even tRNA (Ferbus et al., 1981). To be active, the enzyme has to bind to double-stranded RNA stretches of minimum 50 bp (Minks et al., 1979), and must therefore possess several binding sites: for nucleotide triphosphates, for 2'OH adenosine polynucleotides and for double stranded RNA. The enzyme binds to 2', 5' ADP-Sepharose (Johnston et al., 1980), to poly (rI)(rC)-Agarose (Hovanessian et al., 1977) and to Cibacron Blue-Sepharose (Revel et al., 1981). In different cells, the (2'-5') oligo A **synthetase** activity is in the cytosol (Revel et al., 1981) or in ribosomal salt washes (Dougherty et al., 1980), as well as in the nuclear sap (Nilsen et al., 1982b) and even in large amounts in the nuclear **matrix**. It is notable that cellular RNAs can replace poly (rI)(rC) for activation of the enzyme (Revel et al., 1980) and the **synthetase** may even have a role in Hn RNA processing (Nilsen et al., 1982a). Some (2'-5') oligo A **synthetase** is bound to plasma membranes and can be incorporated in budding virions (Wallach and Revel, 1980). These complex interactions may ensure a localized action of the (2'-5') oligo A system (Nilsen and Baglioni, 1983) and explain its multiple suggested roles in normal and virus-infected cells. The **synthetase** amounts to less than 0.1% of the proteins in IFN-treated cells, and its structure could not be determined directly.

SUMMARY OF THE INVENTION

The present invention concerns human DNA encoding an enzyme having (2'-5') oligo A synthetase activity. One form of the DNA has the nucleotide sequence set forth in FIG. 7A. Another form of the DNA has the sequence of nucleotides 1-1322 set forth in FIG. 7A which overlaps with the sequence of nucleotides 901-1590 set forth in FIG. 7B.

An enzyme having (2'-5') oligo A synthetase activity has the amino acid sequence set forth in FIG. 7A. Another enzyme having (2'-5') oligo A synthetase activity has the sequence of amino acids 1-364 set forth in FIG. 7A which overlaps with the sequence of amino acids 290-400 set forth in FIG. 7B.

A 1.6 kb and 1.8 kb RNA having nucleotide sequences complementary to the nucleotide sequences in FIGS. 7A and 7B have been isolated.

A method of monitoring the response of a patient to an interferon comprises measuring the concentration of (2'-5') oligo A synthetase mRNA in cells or body fluids of the patient by hybridizing to the mRNA DNA complementary thereto.

Antigenic peptides of the present invention have an amino acid sequence contained within the amino acid sequences set forth in FIGS. 7A and 7B. Antibodies raised against these antigenic peptides recognize and immunoprecipitate (2'-5') oligo A synthetase. Also provided are antibodies against all four of the 40 kd, 46 kd, 67 kd and 100 kd forms

of (2'-5') oligo A synthetase, as well as antibodies against one of these forms which does not cross with the other three forms.

A method of monitoring interferon activity in a subject comprises measuring the amount of (2'-5') oligo A synthetase in a cell or body fluid of the subject at predetermined time intervals, determining the differences in the amount of said synthetase in the cell or body fluid of the subject within the different time intervals, and determining therefrom the amount of synthetase in the cell or body fluid of the subject and thereby the interferon activity of the subject. The synthetase may be measured by contacting the synthetase with an antibody of the present invention so as to form a complex therewith and • determining the amount of complex so formed.

DETDESC:

DETD(17)

A method of **monitoring** interferon activity may further comprise, extracting (2'-5') oligo A **synthetase** from a cell or body fluid which has been exposed to interferon, **labeling** the extracted **synthetase** with an identifiable marker to form a **labeled** **synthetase**, contacting the **labeled** **synthetase** with an antibody of the present invention under suitable conditions so as to form a **labeled**-**synthetase**-antibody complex, and detecting the marker in the complex, thereby detecting the **synthetase**. The marker may be .sup.35 S-methionine.

DETDESC:

DETD(18)

A kit for carrying out the method of **monitoring** interferon activity comprises an antibody of the present invention, materials for extracting the **synthetase**, materials for **labeling** the **synthetase**, and materials for detecting the marker and determining the amount of **synthetase**.

DETDESC:

DETD(28)

Both peptides were **synthesized** by the **solid**-phase peptide **synthesis** method of Barany and Merrifield (1980). After purification on Sephadex G25 columns in 2 M acetic acid, the peptides were linked to Keyhole Limpet Hemocyanin (Calbiochem). Fsterification of the NH.sup.2 -terminal arginine of peptide C with p-aminophenylacetic acid allowed to covalently link the peptide to the carrier protein through its amino-terminus (Spirer, et al., 1977). Peptide B was coupled to the carrier protein by ethylene diamine carbodiimide (Hoare and Koshland, 1967).

DETDESC:

DETD(92)

The SphI-SohI fragment of 0.85 kb (FIG. 10) from the genomic 4.2 kb EcoRI fragment (FIG. 9) which contains part of exon 3 of the E16 cDNA 9-21 clone, hybridized in Northern blots with the 1.6, 1.8, 2.7 and 3.6 kb RNAs. However, upstream regions did not. Several experiments allowed to localize the RNA transcriptional start in this fragment. SI nuclease analysis first showed that exon 3 starts about 50 nucleotides upstream of the end of the 9-21 cDNA. A primer extension experiment using an oligonucleotide from the end of the 9-21 cDNA, indicated that the 5' end of the mRNA is about 230 nucleotides from the 5' end of this cDNA. RNA hybridization with riboprobes produced in SP6 (Green et al., 1983) and RNAse digestion indicated two exons of 70 and 110 nucleotides preceding exon 3. By SI nuclease analysis using a probe labeled at the unique HpaI site (FIG. 9), the 5' end of the mRNA was finally located 17 nucleotides upstream from the HpaI site. The sequence of this region is shown in FIG. 11. The location of the transcription initiation site 17 residues before the HpaI site, is supported by the presence of a TATAA box at position -30. A striking feature of the upstream sequences, is the high purine content (69.6%) mostly adenine (58.9%). Run of a homology **matrix** with other known promoter upstream sequences revealed a surprising homology with the human IFN promoters in particular with the sequence of the IFN-beta-1 gene promoter (Degrave et al., 1981). The purine-rich region from -75 to -85 of the IFN-beta-1 promoter, which contains the essential transcription signal described by Zinn et al., (1983), shows 90% homology with the region of the presumed promoter of the (2'-5') oligo A **synthetase** just upstream of the TATAA box (-40 to -50) (FIG. 11). This purine-rich signal is repeated in the IFN-beta-1 promoter in the segment between the TATAA box and the cap site; in this region, which may also have regulatory functions (Nir et al., 1984) the homology between the IFN-beta-1 gene and the (2'-5') oligo A **synthetase** gene is high. In contrast, search for homology with promoters of other genes, such as HLA genes (Malissen et al., 1982; Schamboeck et al., 1983) and the metallothionein II gene (Karin and Richards, 1982) which are activated by IFNs (Fellous et al., 1982; Rosa et al., 1983b; Friedman et al., 1984) showed no apparent sequence relationship in this region of the (2'-5') oligo A **synthetase** gene promoter. Also, no significant homology was seen with the body of the IFN-beta-1 gene.

DETDESC:

DETD(120)

Many observations suggest that the IFN-induced (2'-5') oligo A synthetase involved in two distinct, seemingly opposite, phase of cell growth (cell-cycling and growth inhibition) in addition to its possible role in the antiviral effect (reviewed in Revel, 1984). This may be relevant to the issue of **multiple** (2'-5') oligo A **synthetase** forms. In synchronized cell cultures we have observed that (2'-5') oligo A synthetase behaves as a cell-cycle protein (Mallucci, et al., 1985). Thus, synchronized cultures of Mouse embryo fibroblasts exhibit a sharp rise in (2'-5') oligo A synthetase activity and (2'-5') oligo A synthetase mRNA at the end of the S-phase followed by a rapid disappearance of the RNA and enzyme activity when the cells proceed to G2. Anti-mouse IFN antibodies reduced the (2'-5') oligo A synthetase induction. In this system we also observed that the (2'-5') oligo A synthetase RNA which accumulates in S-phase is a large 4-5 kb transcript different from the 1.7 kb RNA species which accumulated in the same cells when treated with exogenous IFN. This suggests that the S-phase (2'-5') oligo A synthetase is a different form of the enzyme than that in cells growth-arrested by exogenously added IFN. Because of its large mRNA it is likely to be like the 100 kd, a low ds RNA requiring form. Anti-B antibodies also detected the (2'-5') oligo A **synthetase** **multiple** forms in mouse cells.

18. 5,064,754, Nov. 12, 1991, Genomic sequencing method; Randell L. Mills, 435/6, 5, 91.51; 436/94, 173, 174, 175, 501; 935/77, 78 [IMAGE AVAILABLE]

US PAT NO: 5,064,754 [IMAGE AVAILABLE] L7: 18 of 20

ABSTRACT:

A method of determining the nucleotide sequence of a DNA molecule of arbitrary length as a single procedure by sequencing portions of the molecule in a fashion such that the sequence of the 5' end of the succeeding contiguous portion is sequenced as the 3' end of its preceeding portion is sequenced, for all portions, where the order of contiguous portions is determined by the sequence of the DNA molecule. Sequencing of the individual portions is accomplished by generating a family of polynucleotides under conditions which determine that the elements are partial copies of the portion and are of random nucleotide length on the 3' and 5' ends about a dinucleotide which is an internal reference point; determining the base composition and terminal base identity of each element of the family and solving for the sequence by a method of analysis wherein the base composition and terminal base data of each element is used to solve for a single base of the sequence by assigning the base to either the 5' or 3' end of the partial sequence about the internal reference point as the entire sequence of the portion is built up from a dinucleotide.

SUMMARY:

BSUM(18)

In the preferred embodiment of the invention, the polynucleotides are RNA/DNA hybrid molecules generated from the DNA to be sequenced. To form these hybrids, DNA to be sequenced is broken into fragments and each fragment used as a template to form one or more RNA transcript(s). The RNA transcript(s) is then extended on the original intact DNA template with deoxyribonucleotides to form the DNA portion of the hybrid(s). The extension is terminated randomly by addition of dideoxynucleotides to the polymerase reaction. This yields RNA/DNA hybrid **molecules** which are "**random**" in length at the 3' end. The molecules can then be randomized at the 5' (RNA) end preferably by using an RNA exonuclease which under appropriate conditions, degrades the 5' RNA portion. The result of this procedure is a family of polynucleotides having the characteristic set forth above. The "axis" referred to above is the dividing line between the RNA and DNA and it immediately follows the 3' most ribonucleotide of all the hybrid molecules.

DETDESC:

DETD(111)

In the second procedure, which is the preferred method, RNA copies are made without use of a primer. If ribonucleotides are added to the reaction mixture in high concentration in the absence of sigma factor, which controls initiation of RNA polymerization only from special DNA sequences called promoters, then RNA polymerase initiates RNA **synthesis** at **random** points along the DNA template and a free end stimulates initiation at that site. The procedure is carried out in substantially the same way as for primed RNA synthesis except that no primers are added and a concentration of about 2 millimolar ribonucleotides is used.

DETDESC:

DETD(155)

DNA or RNA bands collected as fractions can be **monitored** spectrophotometrically at wavelengths of 260 and 280 nm or by ethidium bromide fluorescent quantitation. See Maniatis, pp. 468-469; 163. The preferred method of ethidium bromide fluorescent quantitation is to use ethidium bromide in the gel or in the anode well at a concentration of about 0.5 ug/ml. See Maniatis, p. 163. Also, it is not necessary to remove ethidium bromide from RNA or DNA to perform primed **synthesis** of Step III. (See Methods of Enzymology; vol. 65, p. 565 (1980)) If the mass **labeled** nucleotides contain in addition a radiolabel, then the bands can be **monitored** by a scintilation counter.

DETDESC:

DETD(235)

In reference to Methods I, two procedures of producing RNA copies of the single stranded restriction fragments are described. See pages 53-54. Procedure A involves using primed RNA **synthesis**; Procedure B involves using RNA polymerase under conditions that allow the enzyme to initiate RNA polymerization randomly on a DNA template with high activity. Both methods may give rise to subsets that have a common 5' end. In this case the simple strategy described below is used to solve for the sequence from the data. But, the replication products may not all have a common 5' end; therefore, the data must be treated as if subsets are made from the loss of nucleotides randomly from the 3' and 5' end of the largest

polynucleotide and the **matrix** method of analysis must be used as described in this discussion.

DETDESC:

DETD(259)

For the preferred method, since the 3' **random** hybrid **molecules** are 5' randomized by a procedure which only removes RNA, the most 3' junction of RNA and DNA relative to the complement of the parent represents the "axis". Furthermore, if one RNA copy is isolated when following the procedure described in Methods I, then each of the polynucleotides generated from subsequent reactions and scanned, yields the solution of one nucleotide. Thus, the ratio of the number of "unknowns" solved to the total number of polynucleotides generated is one. If more than one RNA copy is isolated, then the ratio is x/xR=1/R, where R is the number of RNA copies isolated.

19. 4,863,899, Sep. 5, 1989, Biologically active polypeptides; George J. Todaro, 514/9, 10, 11, 12, 13, 14; 930/10, 120, DIG.821 [IMAGE AVAILABLE]

US PAT NO: 4,863,899 [IMAGE AVAILABLE] L7: 19 of 20

ABSTRACT:
Novel biologically active polypeptides, including a new class of transforming growth factor (TGF) polypeptides, which exhibit cell growth promoting properties are disclosed, as well as a process for isolating the TGF polypeptides from both human and murine cell lines in homogeneous form. Also disclosed are antigenic oligopeptides derived from the TGF polypeptides and antibodies raised therefrom which have application in the detection and treatment of malignancies and oligipeptides which have the ability to bind with cellular growth factor receptors and thus to interfere with transformation of certain cell lines into a cancerous state. Compositions and methods based on the disclosed peptides for detection and treatment of cancer and other proliferative diseases and for cell or tissue growth associated treatment, e.g., wound healing, ulcer therapy and bone loss are also described.

DETDESC:

DETD(37)

The polypeptides and oligopeptides according to the invention as defined by the structural formulas (formulas I through IV) and peptide sequences given above can be prepared by **synthetic** techniques, techniques whereby the peptide is isolated from a naturally occurring source, e.g., cell lines and body fluids, and by techniques employing hybrid DNA technology. For those polypeptides and oligopeptides of the invention containing up to about 50 amino acid residues, conventional **solid** phase peptide **synthesis** is suitably employed. In this general **synthetic** procedure for making peptides, which is described, for example, in U.S. Pat. No. 4,341,761 to Ganfield et al., employs known side-chain protecting groups and conventional polystyrene resins supports--e.g., chloromethylated resins, hydroxymethyl resins or benzhydrylamine resins--to affect the amino acid coupling. For polypeptides containing in excess of about 50 amino acid residues, the process according to the invention for isolating homogeneous TGFs from natural sources (which is described in detail below) can be suitably employed to obtain pure forms of the desired peptide. In this regard, particularly suitable sources of the TGF polypeptides according to the invention include serum-free medium conditioned by retrovirus-transformed Fischer rat embryo fibroblasts, in particular fibroblasts transformed with Snyder-Theilen feline sarcoma virus, Moloney murine sarcoma virus-transformed mouse 3T3 cells and human metastatic melanoma cell lines A2058 and A375. Sources and methods for suitable murine cell lines are described in DeLarco et al. (1980) J. Biol. Chem. 255, pp. 3685-3690 and Ozanne et al. (1980) J. Cell. Physiol. 105, pp. 163-180. Sources and methods for human cell lines are similarly described in Todaro et al. (1980) Proc. Natl. Acad. Sci. USA 77, pp. 5258-5262 and Giard et al. (1973) J. Natl. Cancer Inst. 51, pp. 1417-1423. The isolation process of the invention described below can also be used to obtain TGF polypeptides according to the invention from various body fluids such as urine, serum, plasma, whole blood or cerebrospinal fluid of human or murine subjects carrying malignancies or transformed cells which produce TGF polypeptides. In this regard, a suitable source of TGF polypeptides according to the invention is the urine or other body fluids of mice which have been inoculated with tumor cells (human melanoma or transformed rat) known to produce TGF polypeptides. In all cases the identification and purity of the TGF polypeptide can be monitored by a radioreceptor assay based on receptor cross-reactivity with EGF (see experimental examples below). In techniques utilizing recombinant or hybrid DNA technology, the oligopeptides according to the invention or segments of the polypeptides according to the invention containing up to, for example, 20 amino acids can be used to deduce the codon sequence for single stranded nucleotide (DNA) probes. These nucleotide probes can then be **synthesized** using known **synthetic** techniques and used as a probe to obtain messenger RNA (mRNA) coding for growth factor-type polypeptides in both normal and transformed cells or body fluids containing said peptides. Once messenger RNA is obtained, conventional techniques can be used for reverse transcribing of the mRNA to complementary DNA (cDNA) and subsequent cloning of the cDNA in a suitable vector to obtain expression of the desired polypeptide.

DETDESC:

DETD(123)
was **synthesized** using the **solid**-phase technique of Ohgak et al. (1983) Journal of Immunol. Meth. 57, pp. 171-184. This oligopeptide was then coupled to keyhole limpet hemocyanin in accordance with the procedure of Baron et al. (1982) Cell 28, pp. 395-404, and used to immunize rabbits (Baron et al. (1982) Cell 28, pp. 395-404) and sheep (Lerner (1982) Nature 299, pp. 592-596). Antisera were assayed against peptide by a peroxidase-linked immunoassay (Kirkegaard and Perry Laboratories, Gaithersberg, Md.) and against homogeneous rat TGF (purified according to Example II above), by immunoprecipitation (Bister et al. (1980) J. Virol. 36, pp. 617-621) and Western blotting techniques (Burnett (1981) Analyt. Biochem. 112, pp. 195-203). Binding of .sup.125 I-labeled rat TGF and mouse EGF (Bethesda Research Labs, Bethesda, Md.) to A431 cells grown in 96-well microtiter plates was as described in Pross et al. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, pp. 3918-3921.

DETDESC:

DETD(131)

The chemical **synthesis** of rat TGF, having the chemical formula given in Example II, was performed manually by the stepwise **solid**-phase approach according to the general principles described by Merrifield (1963) J. Amer. Chem. Soc. 85, pp. 2149-2156. The differential acid-labile protecting group strategy was adopted for this **synthesis** with the conventional combination of tertbutyloxycarbonyl for N-amino terminus and benzyl alcohol derivatives for the side chains. A more acid stable benzyl ester linkage that anchored protected amino acids to the polymeric **support** was used to minimize loss of peptides during the repetitive acid treatments (Mitchell et al. (1976) J. Amer. Chem. Soc. 92, pp. 7357-7362). Complete deprotection and removal of peptide from the resin was by the low-high HF method of Tam et al. (1983) Tetrahedron Lett. 23, pp. 4435-4438, which differed from the conventional HF deprotection method and removed benzyl protecting groups by the S.sub.N 2 mechanism in dilute HF solution to minimize serious side reactions due to carbocations generated in the conventional S.sub.N 1 deprotection method. Furthermore, it is also designed to reduce many cysteinyl side reactions that often hamper the **synthesis** of proteins containing **multiple** disulfide linkages.

DETDESC:

DETD(133)

Under reducing or nonreducing conditions, the purified **synthetic** rTGF was found to give a single band with an apparent molecular weight of 7000 on SDS-PAGE electrophoresis. Amino acid analysis by 6N HCl and enzymatic hydrolysis provided the expected theoretical molar ratio of the proposed sequence. No free thiol was detected by Ellman's method of sulhydryl determination on **synthetic** rTGF, but upon thiolytic reduction, the expected theoretical value of six cysteines was obtained. These findings **support** the conclusion that **synthetic** rTGF is a single chain polypeptide containing six cysteines in disulfide linkages, which is in agreement with the expected chemical properties of the natural rTGF. Additionally, **synthetic** rTGF coeluted with the natural rTGF as a single symmetrical peak in C.sub.18 reverse phase HPLC.

DETDESC:

DETD(194)

Peptides, corresponding to amino acids from portions of rTGF amino acid sequence, described above in Example II, were **synthesized** commercially (Peninsula Labs) by the standard **solid** phase technique of Ohgak et al. (1983) Journal of Immunol. Meth. 57, pp. 171-184. If necessary, peptides were purified by reverse phase high performance liquid chromatography (HPLC) prior to use. The sequences used were:

DETDESC:

DETD(251)

Synthetic rat TGF was prepared as described above in Example V. Purity of the protein was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, amino acid analysis, and reverse phase high-performance liquid chromatography. The biological activity of TGF preparations and **synthetic** TGF was **monitored** by means of an EGF radioreceptor assay. (Todaro et al. (1976) Nature 264, 26.) Bone resorption was assessed by measuring the release of .sup.45 Ca from previously **labeled** fetal rat long bones. Pregnant rats at the 18th day of gestation were injected with 200 .mu.Ci of .sup.45 Ca. (Raisz (1975) J. Clin. Invest. 44, 103.) The mothers were killed on the 19th day of gestation, and the fetuses were removed. The mineralized shafts of the radii and ulnae were dissected free of surrounding tissue and cartilage and placed in organ culture. The bones were incubated in BGJb medium (Irvine Scientific) for 24 hours at 37.degree. C. in a humidified atmosphere of 5 percent of CO.sub.2 and 95 percent air to allow for the exchange of loosely complexed .sup.45 Ca. The bones were then cultured for 48 to 120 hours in BGJb medium supplemented with 5 percent fetal calf serum (KC Biologicals) containing control or test substances. Bone-resorbing activity was measured as the percentage of total .sup.45 Ca released into the medium and was expressed as a treated-to-control ratio. Statistical significance was determined with Student's t test for unpaired data.

20. 4,816,561, Mar. 28, 1989, Biologically active polypeptides; George J. Todaro, 530/324, 325, 326, 327; 930/120, DIG.811, DIG.821 [IMAGE AVAILABLE]

US PAT NO: 4,816,561 [IMAGE AVAILABLE] L7: 20 of 20

ABSTRACT:
Novel biologically active polypeptides, including a new class of transforming growth factor (TGF) polypeptides, which exhibit cell growth promoting properties are disclosed, as well as a process for isolating the TGF polypeptides from both human and murine cell lines in homogeneous form. Also disclosed are antigenic oligopeptides derived from the TGF polypeptides and antibodies raised therefrom which have application in the detection and treatment of malignancies and oligipeptides which have the ability to bind with cellular growth factor receptors and thus to interfere with transformation of certain cell lines into a cancerous state. Compositions and methods based on the disclosed peptides for detection and treatment of cancer and other proliferative diseases and

for cell or tissue growth associated treatment, e.g., wound healing, ulcer therapy and bone loss are also described.

SUMMARY:

BSUM(68)

The polypeptides and oligopeptides according to the invention as defined by the structural formulas (formulas I through IV) and peptide sequences given above can be prepared by **synthetic** techniques, techniques whereby the peptide is isolated from a naturally occurring source, e.g., cell lines and body fluids, and by techniques employing hybrid DNA technology. For those polypeptides and oligopeptides of the invention containing up to about 50 amino acid residues, conventional **solid** phase peptide **synthesis** is suitably employed. In this general **synthetic** procedure for making peptides, which is described, for example, in U.S. Pat. No. 4,341,761 to Ganfield et al., employs known side-chain protecting groups and conventional polystyrene resins supports--e.g., chloromethylated resins, hydroxymethyl resins or benzhydrylamine resins--to affect the amino acid coupling. For polypeptides containing in excess of about 50 amino acid residues, the process according to the invention for isolating homogeneous TGFs from natural sources (which is described in detail below) can be suitably employed to obtain pure forms of the desired peptide. In this regard, particularly suitable sources of the TGF polypeptides according to the invention include serum-free medium conditioned by retrovirus-transformed Fischer rat embryo fibroblasts, in particular fibroblasts transformed with Snyder-Theilen feline sarcoma virus, Moloney murine sarcoma virus-transformed mouse 3T3 cells and human metastatic melanoma cell lines A2058 and A375. Sources and methods for suitable murine cell lines are described in DeLarco et al. (1980) J. Biol. Chem. 255, pp. 3685-3690 and Ozanne et al. (1980) J. Cell. Physiol. 105, pp. 163-180. Sources and methods for human cell lines are similarly described in Todaro et al. (1980) Proc. Natl. Acad. Sci. USA 77, pp. 5258-5262 and Giard et al. (1973) J. Natl. Cancer Inst. 51, pp. 1417-1423. The isolation process of the invention described below can also be used to obtain TGF polypeptides according to the invention from various body fluids such as urine, serum, plasma, whole blood or cerebrospinal fluid of human or murine subjects carrying malignancies or transformed cells which produce TGF polypeptides. In this regard, a suitable source of TGF polypeptides according to the invention is the urine or other body fluids of mice which have been inoculated with tumor cells (human melanoma or transformed rat) known to produce TGF polypeptides. In all cases the identification and purity of the TGF polypeptide can be monitored by a radioreceptor assay based on receptor cross-reactivity with EGF (see experimental examples below). In techniques utilizing recombinant or hybrid DNA technology, the oligopeptides according to the invention or segments of the polypeptides according to the invention containing up to, for example, 20 amino acids can be used to deduce the codon sequence for single stranded nucleotide (DNA) probes. These nucleotide probes can then be **synthesized** using known **synthetic** techniques and used as a probe to obtain messenger RNA (mRNA) coding for growth factor-type polypeptides in both normal and transformed cells or body fluids containing said peptides. Once messenger RNA is obtained, conventional techniques can be used for reverse transcribing of the mRNA to complementary DNA (cDNA) and subsequent cloning of the cDNA in a suitable vector to obtain expression of the desired polypeptide.

DETDESC:

DETD(65)
was **synthesized** using the **solid**-phase technique of Ohgak et al. (1983) Journal of Immunol. Meth. 57, pp. 171-184. This oligopeptide was then coupled to keyhole limpet hemocyanin in accordance with the procedure of Baron et al. (1982) Cell 28, pp. 395-404, and used to immunize rabbits (Baron et al. (1982) Cell 28, pp. 395-404) and sheep (Lerner (1982) Nature 299 pp. 592-596). Antisera were assayed against peptide by a peroxidase-linked immunoassay (Kirkegaard and Perry Laboratories, Gaithersberg, MD) and against homogeneous rat TGF (purified according to Example II above), by immunoprecipitation (Bister et al. (1980) J. Virol. 36, pp. 617-621) and Western blotting techniques (Burnett (1981) Analyt. Biochem. 112, pp. 195-203). Binding of .sup.125 I-labeled rat TGF and mouse EGF (Bethesda Research Labs, Bethesda, MD) to A431 cells grown in 96-well microtiter plates was as described in Pross et al. (1977) Proc. Natl. Acad. Sci. USA 74, pp. 3918-3921.

DETDESC:

DETD(73)

The chemical **synthesis** of rat TGF, having the chemical formula given in Example II, was performed manually by the stepwise **solid**-phase approach according to the general principles described by

DETDESC:

DETD(74)

Merrifield (1963) J. Amer. Chem. Soc. 85, pp. 2149-2156. The differential acid-labile protecting group strategy was adopted for this **synthesis** with the conventional combination of tertbutyloxycarbonyl for N-amino terminus and benzyl alcohol derivatives for the side chains. A more acid stable benzyl ester linkage that anchored protected amino acids to the polymeric **support** was used to minimize loss of peptides during the repetitive acid treatments (Mitchell et al. (1976) J. Amer. Chem. Soc. 92, pp. 7357-7362). Complete deprotection and removal of peptide from the resin was by the low-high HF method of Tam et al. (1983) Tetrahedron Lett. 23, pp. 4435-4438, which differed from the conventional HF deprotection method and removed benzyl protecting groups by the S.sub.N 2 mechanism in dilute HF solution to minimize serious side reactions due to carbocations generated in the conventional S.sub.N 1 deprotection method. Furthermore, it is also designed to reduce many cysteinyl side reactions that often hamper the **synthesis** of proteins containing **multiple** disulfide linkages.

DETDESC:

DETD(76)

Under reducing or nonreducing conditions, the purified **synthetic** rTGF was found to give a single band with an apparent molecular weight of 7000 on SDS-PAGE electrophoresis. Amino acid analysis by 6N HCl and enzymatic hydrolysis provided the expected theoretical molar ratio of the proposed sequence. No free thiol was detected by Ellman's method of sulhydryl determination on **synthetic** rTGF, but upon thiolytic reduction, the expected theoretical value of six cysteines was obtained. These findings **support** the conclusion that **synthetic** rTGF is a single chain polypeptide containing six cysteines in disulfide linkages, which is in agreement with the expected chemical properties of the natural rTGF. Additionally, **synthetic** rTGF coeluted with the natural rTGF as a single symmetrical peak in C.sub.18 reverse phase HPLC.

DETDESC:

DETD(137)

Peptides, corresponding to amino acids from portions of rTGF amino acid sequence, described above in Example II, were **synthesized** commercially (Peninsula Labs) by the standard **solid** phase technique of Ohgak et al. (1983) Journal of Immunol. Meth. 57, pp. 171-184. If necessary, peptides were purified by reverse phase high performance liquid chromatography (HPLC) prior to use.

DETDESC:

DETD(195)

Synthetic rat TGF was prepared as described above in Example V. Purity of the protein was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, amino acid analysis, and reverse phase high-performance liquid chromatography. The biological activity of TGF preparations and **synthetic** TGF was **monitored** by means of an EGF radioreceptor assay. (Todaro et al. (1976) Nature 264, 26.) Bone resorption was assessed by measuring the release of .sup.45 Ca from previously **labeled** fetal rat long bones. Pregnant rats at the 18th day of gestation were injected with 200 .mu.Ci of .sup.45 Ca. (Raisz (1975) J. Clin. Invest. 44, 103.) The mothers were killed on the 19th day of gestation, and the fetuses were removed. The mineralized shafts of the radii and ulnae were dissected free of surrounding tissue and cartilage and placed in organ culture. The bones were incubated in BGJb medium (Irvine Scientific) for 24 hours at 37.degree. C. in a humidified atmosphere of 5 percent of CO.sub.2 and 95 percent air to allow for the exchange of loosely complexed .sup.45 Ca. The bones were then cultured for 48 to 120 hours in BGJb medium supplemented with 5 percent fetal calf serum (KC Biologicals) containing control or test substances. Bone-resorbing activity was measured as the percentage of total .sup.45 Ca released into the medium and was expressed as a treated-to-control ratio. Statistical significance was determined with Student's t test for unpaired data.

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(FILE 'USPAT' ENTERED AT 12:25:23 ON 14 APR 1998)
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L3 44 S L2 AND (MONITOR### (4A) SYNTHES###)
L4 35 S L2 AND (MONITOR### (3A) SYNTHES###)
L5 7 S L3 AND (LABEL#### (P)MONITOR######)
L6 22 S L2 AND (LABEL#### (P)MONITOR###### (P) SYNTHE####)
L7 20 S L6 NOT L5

=> s l7 and HPLC

23757 HPLC
L8 11 L7 AND HPLC

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113286 LABEL####
256602 MONITOR######
322138 SYNTHE####
23757 HPLC
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L9 1 L2 AND (LABEL#### (P)MONITOR###### (P) SYNTHE#### (P) HPLC)

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1. 5,635,602, Jun. 3, 1997, Design and synthesis of bispecific DNA-antibody conjugates; Charles R. Cantor, et al., 530/391.1, 387.3, 391.5, 391.9; 536/23.1 [IMAGE AVAILABLE]

US PAT NO: 5,635,602 [IMAGE AVAILABLE] L9: 1 of 1

ABSTRACT:
The invention relates to bis-protein-DNA conjugates. A protein having an antigen specific binding activity is covalently linked to each end of a derivatized DNA molecule. The bis-protein-DNA conjugates can be used for immunoassays and measuring distances between proteins at up to 3.4 .ANG. resolution. The invention also relates to methods of synthesizing these bis-protein-DNA conjugates. Synthesis of the conjugates entails derivatizing the 5' or 3' end of a DNA oligonucleotide and covalently linking that DNA to a protein. The DNA can be indirectly conjugated to an antibody or Fab' fragment, using a avidin/streptavidin-biotin linkage.

The conjugates of the invention can be used in immunoassays and PCR assays.

DETDESC:

DETD(4)

A special advantage of using DNA as a molecular scaffold for constructing **arrays** of other **molecules** is that one is not limited to DNA with 2 ends. For example, 3 and 4 ended junctions have been made and they can form the basis for an endless array of more complex structures. Therefore, tri- and tetra-specific antibodies can be prepared using other higher order DNA structures such as Holliday junctions in conjunction with the methods provided below for preparing bi-specific antibodies. In addition, numerous proteins are known with very specific DNA binding sites. Such proteins can augment and enhance the methods for starting with DNA and producing a specific assembly of other molecules.

DETDESC:

DETD(86)

Activated thiol derivatives were prepared essentially according to the method of Brennan et al. (1985). F(ab').sub.2 fragments, obtained by pepsin cleavage (Parham, 1983) of 1 to 5 mg of polyclonal affinity-purified goat anti-mouse IgG (1 mg), monoclonal mouse IgG1 anti-human MHC class I (GA2) (3 mg) or anti-human CD4 (anti-Leu3a) (4 mg) were incubated overnight at room temperature in buffer containing 0.1M sodium phosphate pH 6.8, 2 mM 2-mercaptoethylamine-HCl (Pierce Chemical Co.) and 1 mM EDTA with or without 10 mM sodium arsenite (for free thiol group protection) at a protein concentration of 3 mg/ml. The next day, excess **solid** 5'dithobis(2-nitrobenzoic acid) (Ellman's reagent) was added to the solutions to a concentration of 10 mM. After incubation for 3 h at 25.degree. C., the reaction solutions were desalted and exchanged into buffer containing 0.1M sodium phosphate pH 6.8 and 1 mM EDTA using 6,000 molecular weight exclusion polyacrylamide columns. Five mg of mouse IgG2a F(ab').sub.2 anti-human TCR idiotype (T40/25) on HPB-ALL cells, was reduced to Fab' at 37.degree. C. by treatment for 90 min with 1 ml of 50 mM 2-mercaptoethylamine.HCl, 5 mM EDTA, pH 6.0 (according to the manufacturer's instructions, Pierce Chemical Co.). The sample was then desalted, reacted with Ellman's reagent (added as a **solid** to 10 mM) for 3 hr. at 25.degree. C., and then desalted again into 0.1M sodium phosphate pH 6.8 and 1 mM EDTA as above. In addition, some derivatives were **synthesized** using FITC- and TRITC-conjugated F(ab').sub.2 as the starting material. The dye molecules were usually attached via .epsilon.-amino groups of free lysine residues, and thus would not interfere with these disulfide exchange reactions.

DETDESC:

DETD(89)

Purified, complementary 5'-end-thiolated oligonucleotides (SEQ ID NOS: 1 and 2) were separately mixed with equimolar amounts of thionitrobenzoate-derivatized antibody fragments and allowed to react separately for 16 h at room temperature. If crude derivatized oligonucleotide (purity varied from 70 to 90%) was used, the reaction was carried out in two-fold molar DNA excess. The concentration of Fab' in the reaction solution was approximately 1 to 2 mg/ml. Released thionitrobenzoate, as a consequence of disulfide exchange, could be **monitored** by absorbance at 412 nm (.epsilon..sub.412 =1.36.times.10.sup.4 cm.sup.-1.M.sup.-1) and observed as a yellow color in the reaction solution. The reaction mixtures were then exchanged into 20 mM TrisCl and 1 mM EDTA and purified by anion exchange **HPLC** (7.5 cm.times.7.5 mm DEAE-3SW, Toso Haas) at a flow rate of 1 ml/min with elution by a 0-800 mM NaCl gradient. Fractions were analyzed with 7.5% SDS-PAGE (Laemmli, 1970) and silver staining (Biorad). Purified Fab'-single-stranded DNA conjugates were then mixed and allowed to anneal at 0.degree. C. SDS-PAGE gels were also stained with ethidium bromide (unfixed) and Coomassie (fixed) stains. To confirm the presence of DNA in putative conjugate fractions, treatments with DNHAse I (Sigma Chemical Co.) were performed. Non-denaturing PAGE analysis (Davis, 1964) was also performed on single and double-stranded conjugates. The entire **synthetic** scheme is diagrammed in FIG. 8. Dye **labeling**, which is not illustrated here, may in many cases only be done after Fab'-DNA conjugation, as dye **labeling** introduces significant heterogeneity in both the reaction and the separation (unpublished observations). Dye-**labeled** antibody preparations must be purified and characterized before carrying out such conjugation reactions.

DETDESC:

DETD(198)

Huse, W. D., Sastry, L., Iverson, S. A., Kang, A. S., Alting-Mees, M., Burton, D. R., Benkovic, S. J., and Lerner, R. A. (1989). Generation of a large **combinatorial** **library** of the immunoglobulin repertoire in phage lambda. Science 246, 1275-1281.

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ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
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U.S. Patent & Trademark Office LOGOFF AT 13:03:56 ON 14 APR 1998

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The modem is not responding to modem commands.

Trying 01181...Open

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PLEASE ENTER HOST PORT ID:x
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PASSWORD:
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E5	USPAT	10	LAM1/BI
E6	USPAT	3	LAM1-1/BI
E7	USPAT	1	LAM1-10/BI
E8	USPAT	2	LAM1-14/BI
E9	USPAT	2	LAM1-15/BI
E10	USPAT	1	LAM1-2/BI
E11	USPAT	4	LAM1-3/BI
E12	USPAT	2	LAM1-5/BI

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E3	USPAT	0	--> LAM, K?/IN
E4	USPAT	3	LAM, KA DUK/IN
E5	USPAT	1	LAM, KAH H P/IN
E6	USPAT	1	LAM, KAH HONG PAUL/IN
E7	USPAT	1	LAM, KAI Y/IN
E8	USPAT	1	LAM, KAM HOONG/IN
E9	USPAT	1	LAM, KAM KHEONG/IN
E10	USPAT	1	LAM, KAM YAU/IN
E11	USPAT	1	LAM, KEI B/IN
E12	USPAT	6	LAM, KEN/IN

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E14	USPAT	1	LAM, KHIET THANH/IN
E15	USPAT	1	LAM, KIM V/IN

E16 USPAT 1 LAM, KIN C/IN
E17 USPAT 15 LAM, KIN S/IN
E18 USPAT 2 LAM, KIN SANG/IN
E19 USPAT 1 LAM, KIN SING/IN
E20 USPAT 1 LAM, KING S/IN
E21 USPAT 2 LAM, KIRBY/IN
E22 USPAT 2 LAM, KIT M/IN
E23 USPAT 3 LAM, KIT S/IN
E24 USPAT 2 LAM, KIT SANG/IN

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E33 USPAT 1 LAM, LAWRENCE K/IN
E34 USPAT 2 LAM, LAWRENCE K C/IN
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E36 USPAT 1 LAM, LESLIE/IN

=> s e22-e24

2 "LAM, KIT M"/IN
3 "LAM, KIT S"/IN
2 "LAM, KIT SANG"/IN
L1 7 ("LAM, KIT M"/IN OR "LAM, KIT S"/IN OR "LAM, KIT SANG"/IN)

=> s l1 and hplc

23757 HPLC
L2 3 L1 AND HPLC

=> s l3 and electrophoresis

'L3' NOT FOUND

=> s l2 and electrophoresis

18460 ELECTROPHORESIS
L3 0 L2 AND ELECTROPHORESIS

=> d l2 kwic

US PAT NO: 5,650,489 [IMAGE AVAILABLE] L2: 1 of 3
INVENTOR: **Kit Sang Lam**, Tucson, AZ
Sydney E. Salmon, Tucson, AZ

DRAWING DESC:

DRWD(4)

FIG. 3. Chromatograms (C.sub.18 reverse phase **HPLC**, Vydac) of random tetrapeptides (X-X-X-W where X=S, A, or V) synthesized by: (A) new approach (see text), and (B) standard. . .

DETDESC:

DETD(204)

Both peptide libraries were analyzed on a C-18 reverse phase **HPLC** chromatography column (Vydac) to demonstrate the number of peptide species in the library (number of peaks), relative concentration of peptides(area. . .

DETDESC:

DETD(278)

Peptide . . . Ci/mmole, New England Nuclear, Boston, Mass.). The [.sup.3 H]Ac-v-mos product, which was separated from unreacted v-mos peptide with reverse phase **HPLC**, had a specific activity of 2.50 Ci/mmole. The binding affinity of [.sup.3 H]Ac-v-mos for anti-v-mos MAb (=10 .mu.g/ml) was measured. . .

DETDESC:

DETD(298)

After . . . supernatant was filtered off, lyophilized and re-dissolved to equal volumes of MeOH (0.3 ml). The products were analyzed on the **HPLC**.

DETDESC:

DETD(300)

Analysis by **HPLC** clearly showed no tripeptide release upon VIS irradiation in water, and almost complete release of tripeptide upon UV irradiation for. . .

DETDESC:

DETD(301)

In . . . peptide i in water was cleaved to a single product. In some cases, two products were observed to elute from **HPLC**. At longer exposure times (UV), interconversion between the two products was observed in at least a few cases.

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'L3' HAS NO ANSWERS
L1 7 SEA FILE=USPAT ("LAM, KIT M"/IN OR "LAM, KIT S"/IN OR "LAM , K
IT SANG"/IN)
L2 3 SEA FILE=USPAT L1 AND HPLC
L3 0 SEA FILE=USPAT L2 AND ELECTROPHORESIS

=> d l3 1 -3 cit ab kwic

'L3' HAS NO ANSWERS
L1 7 SEA FILE=USPAT ("LAM, KIT M"/IN OR "LAM, KIT S"/IN OR "LAM , K
IT SANG"/IN)
L2 3 SEA FILE=USPAT L1 AND HPLC
L3 0 SEA FILE=USPAT L2 AND ELECTROPHORESIS

=> d l2 1 -3 cit ab kwic

1. 5,650,489, Jul. 22, 1997, Random bio-oligomer library, a method of synthesis thereof, and a method of use thereof; **Kit Sang Lam**, et al., 530/334; 435/183; 436/86, 544; 530/300, 333, 344, 350, 806, 812, 817 [IMAGE AVAILABLE]

US PAT NO: 5,650,489 [IMAGE AVAILABLE] L2: 1 of 3

ABSTRACT:
The instant invention provides a library of bio-oligomers of defined size and known composition, in which the library contains all of the possible sequences of the bio-oligomers, and a method of synthesis thereof. The bio-oligomers of the library may be peptides, nucleic acids, or a combination of the foregoing. The instant invention also provides methods to identify bio-oligomers from a library that demonstrate desired characteristics such as binding, bioactivity and catalytic activity. Thus the instant invention provides a unique and powerful method to identify a useful bio-oligomer sequences from a library more quickly than current state-of-the-art technology allows. Effector molecules for use in treatment or diagnosis of disease are also provided.
INVENTOR: **Kit Sang Lam**, Tucson, AZ
Sydney E. Salmon, Tucson, AZ

DRAWING DESC:

DRWD(4)

FIG. 3. Chromatograms (C.sub.18 reverse phase **HPLC**, Vydac) of random tetrapeptides (X-X-X-W where X=S, A, or V) synthesized by: (A) new approach (see text), and (B) standard. . .

DETDESC:

DETD(204)

Both peptide libraries were analyzed on a C-18 reverse phase **HPLC** chromatography column (Vydac) to demonstrate the number of peptide species in the library (number of peaks), relative concentration of peptides(area. . .

DETDESC:

DETD(278)

Peptide . . . Ci/mmole, New England Nuclear, Boston, Mass.). The [.sup.3 H]Ac-v-mos product, which was separated from unreacted v-mos peptide with reverse phase **HPLC**, had a specific activity of 2.50 Ci/mmole. The binding affinity of [.sup.3 H]Ac-v-mos for anti-v-mos MAb (=10 .mu.g/ml) was measured. . .

DETDESC:

DETD(298)

After . . . supernatant was filtered off, lyophilized and re-dissolved to equal volumes of MeOH (0.3 ml). The products were analyzed on the **HPLC**.

DETDESC:

DETD(300)

Analysis by **HPLC** clearly showed no tripeptide release upon VIS irradiation in water, and almost complete release of tripeptide upon UV irradiation for. . .

DETDESC:

DETD(301)

In . . . peptide i in water was cleaved to a single product. In some cases, two products were observed to elute from **HPLC**. At longer exposure times (UV), interconversion between the two products was observed in at least a few cases.

1. 5,650,489, Jul. 22, 1997, Random bio-oligomer library, a method of synthesis thereof, and a method of use thereof; **Kit Sang Lam**, et al., 530/334; 435/183; 436/86, 544; 530/300, 333, 344, 350, 806, 812, 817 [IMAGE AVAILABLE]

US PAT NO: 5,650,489 [IMAGE AVAILABLE] L2: 1 of 3

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2. 5,635,598, Jun. 3, 1997, Selectively cleavabe linners based on iminodiacetic acid esters for solid phase peptide synthesis; Michal Lebl, et al., 530/334, 343, 345 [IMAGE AVAILABLE]

US PAT NO: 5,635,598 [IMAGE AVAILABLE] L2: 2 of 3

ABSTRACT:
The present invention is directed to linkers based on ester bond linkages, especially iminodiacetic acid ester bond linkages, for use in solid phase peptide synthesis. In particular, the invention is directed to cleavable linkers that can release peptide from the solid phase support under relatively mild conditions by formation of a diketopiperazine or other cyclic structure, such that the cyclic structure remains on the solid phase support, and, in a second cleavage, under more stringent conditions of high pH. The invention is further directed to solid phase supports prepared with multiple cleavable linkers, including a linker that is cleaved by formation of a cyclic product. One such second linker is an ester of hydroxymethylbenzoic acid, or esters formed by carboxy groups of aspartic or glutamic acid.
INVENTOR: Michal Lebl, Oro Valley, AZ
Viktor Krchnak, Oro Valley, AZ
Petr Kocis, Oro Valley, AZ
Kit S. Lam, Tucson, AZ

SUMMARY:

BSUM(54)

. . .
1,2-ethanedithiol
HOBt 1-hydroxybenzotriazole
IDA iminodiacetic acid
Npys 2-nitropyridylsulfenyl
PA, PAO propanyl amine (or amide) ester
PAOH propanol amine (or amide)
RP-**HPLC** reversed-phase high performance liquid chromatography
SPPS solid-phase peptide synthesis
TFA trifluoroacetic acid
TG TentaGel (resin)
Z benzyloxycarbonyl

DETDESC:

DETD(88)

Amino . . . solvents. UV/VIS absorption spectra were recorded on a Hewlett-Packard HP8452A Diode-Array spectrophotometer using 1-cm quartz cuvette. Both analytical and preparative **HPLC** were carried out on a modular Spectra Physics system using Vydac (0.46.times.250 mm, 5 .mu.m, flow 1 ml/min) and Vydac. . .

DETDESC:

DETD(129)

Double . . . of the reaction whereby the release is accompanied by the generation of hexahydropyrrolo(1,2-a)pyrazine-1,4-dione (HHPPD) is shown in Scheme 2, supra. **HPLC** analysis of the released peptides revealed that in addition to the above mechanism there was unexpectedly a second mechanism of. . .

DETDESC:

DETD(139)

Leu-enkephalin . . . 8.3 yielded 65 mmol of peptide per gram of resin. The second release at pH 13.2 yielded 62 mmol/g. Analytical **HPLC** showed the same peak for both released peptides. The purity of both released peptides was greater than 98%.

DETDESC:

DETD(150)

This . . . of a pentapeptide as described above. The product was cleaved from both arms and its purity was determined by analytical **HPLC**, the structure was confirmed by MS.

DETDESC:

DETD(153)

Instrumentation. . . . 1-cm quartz cuvette. Amino acid analyses were carried out on a D-500 (Durrum Corp., USA) system. Both analytical and preparative **HPLC** were carried out on a modular Spectra Physics system using Vydac (0.46.times.250 mm, 5 .mu.m, flow 1 ml/min) and Vydac. . .

3. 5,510,240, Apr. 23, 1996, Method of screening a peptide library; **Kit S. Lam**, et al., 435/7.1, 4, 18, 23, 24; 436/86, 89, 90, 501, 518, 524, 528, 531; 530/350, 387.1 [IMAGE AVAILABLE]

US PAT NO: 5,510,240 [IMAGE AVAILABLE] L2: 3 of 3

ABSTRACT:
The instant invention provides a library of bio-oligomers of defined size and known composition, in which the library contains all of the possible sequences of the bio-oligomers, and a method of synthesis thereof. The bio-oligomers of the library may be peptides, nucleic acids, or a combination of the foregoing. The instant invention also provides methods to identify bio-oligomers from a library that demonstrate desired characteristics such as binding, bioactivity and catalytic activity. Thus the instant invention provides a unique and powerful method to identify a useful bio-oligomer sequences from a library more quickly than current state-of-the-art technology allows. Effector molecules for use in treatment or diagnosis of disease are also provided.
INVENTOR: **Kit S. Lam**, Tucson, AZ
Sydney E. Salmon, Tucson, AZ

DRAWING DESC:

DRWD(4)

FIG. 3. Chromatograms (C.sub.18 reverse phase **HPLC**, Vydac) of random tetrapeptides (X-X-X-W (SEQ. ID NOS: 1-27) where X=S, A, or V) synthesized by: (A) new approach (see. . .

DETDESC:

DETD(174)

Both peptide libraries were analyzed on a C-18 reverse phase **HPLC** chromatography column (Vydac) to demonstrate the number of peptide species in the library (number of peaks), relative concentration of peptides. . .

DETDESC:

DETD(244)

Peptide . . . Ci/mmole, New England Nuclear, Boston, Mass.). The [.sup.3 H]Ac-v-mos product, which was separated from unreacted v-mos peptide with reverse phase **HPLC**, had a specific activity of 2.50 Ci/mmole. The binding affinity of [.sup.3 H]Ac-v-mos for anti-v-mos MAb (=10 .mu.g/ml) was measured. . .

DETDESC:

DETD(263)

After . . . supernatant was filtered off, lyophilized and re-dissolved to equal volumes of MeOH (0.3 ml). The products were

analyzed on the **HPLC**.

DETDESC:

DETD(265)

Analysis by **HPLC** clearly showed no tripeptide release upon VIS irradiation in water, and almost complete release of tripeptide upon UV irradiation for. . .

DETDESC:

DETD(266)

In . . . peptide i in water was cleaved to a single product. In some cases, two products were observed to elute from **HPLC**. At longer exposure times (UV), interconversion between the two products was observed in at least a few cases.

=> s 5510240/pn

L4 1 5510240/PN

=> s l4 and sds-page

12483 SDS
107024 PAGE
5602 SDS-PAGE
(SDS(W)PAGE)
L5 0 L4 AND SDS-PAGE

=> s l4 and sds

12483 SDS
L6 1 L4 AND SDS

=> d kwic

US PAT NO: **5,510,240** [IMAGE AVAILABLE] L6: 1 of 1

DETDESC:

DETD(131)

MTT . . . bath sonicator for 120 seconds. The extraction solution comprises 50% (v/v) N,N-dimethylformamide in a 20% (w/v) solution of sodium dodecylsulfate (**SDS**) adjusted to pH 4.7 with acetic-HCl acid as described by Hansen, et al. (1989, J. Immunol. Methods 119:203). This treatment. . .

=> s l4 and ion exchange

157205 ION
148007 EXCHANGE
41545 ION EXCHANGE
(ION(W)EXCHANGE)
L7 0 L4 AND ION EXCHANGE

=> s l4 and size

840305 SIZE
L8 1 L4 AND SIZE

=> d kwic

US PAT NO: **5,510,240** [IMAGE AVAILABLE] L8: 1 of 1

ABSTRACT:
The instant invention provides a library of bio-oligomers of defined **size** and known composition, in which the library contains all of the possible sequences of the bio-oligomers, and a method of. . .

DETDESC:

DETD(145)

Alternatively, . . . of peptide. With state of the art peptide sequencer, only 5-10 pmole is required for adequate sequencing. Therefore, one standard **size**, single PAM resin support of 100 .mu.m diameters contains more than an adequate amount of peptide for sequencing.

DETDESC:

DETD(247)

The . . . the other hand, the negative beads selected randomly did not shown any common amino acid sequence pattern. Although the sample **size** is limited, the chi-square goodness of fit statistic for the sequences from the negative beads was not significant (x.sup.2 =18.27,. . .

=> s l4 and (gel or gelfiltration)

150765 GEL
119 GELFILTRATION
L9 1 L4 AND (GEL OR GELFILTRATION)

=> d kwic

US PAT NO: **5,510,240** [IMAGE AVAILABLE] L9: 1 of 1

DETDESC:

DETD(114)

(iv) Stimulation of cytokine release may be assayed by adding a single cell suspension immobilized in a semi-solid matrix, e.g., agarose **gel**. Where a bio-oligomer of the invention induces release of cytokine, e.g., lymphokine, growth factor, hormone, etc., presence of the cytokine. . .

DETDESC:

DETD(137)

In . . . color or fluorescence, may be formed in a semi-solid matrix. A library is layered in a semi-solid matrix, e.g., agarose **gel**, and a chromogenic or other indicator substrate is added. Where a bio-oligomer/solid phase support shows the desirable enzyme activity, a. . .

DETDESC:

DETD(159)

The . . . liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica **gel**, magnesium carbonate, magnesium stearate, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and. . .

=> s l4 and filtration

135959 FILTRATION
L10 0 L4 AND FILTRATION

=> s l4 and sephadex

11462 SEPHADEX
L11 0 L4 AND SEPHADEX

=> s librar### and heterogenous

16574 LIBRAR###
2879 HETEROGENOUS
L12 213 LIBRAR### AND HETEROGENOUS

=> s librar### (p) heterogenous

16574 LIBRAR###
2879 HETEROGENOUS
L13 2 LIBRAR### (P) HETEROGENOUS

=> d cit ab kwic

1. 5,512,463, Apr. 30, 1996, Enzymatic inverse polymerase chain reaction library mutagenesis; Willem P. C. Stemmer, 435/91.2, 6, 69.1; 536/24.1, 24.33; 935/77, 78 [IMAGE AVAILABLE]

US PAT NO: 5,512,463 [IMAGE AVAILABLE] L13: 1 of 2

ABSTRACT:
This invention discloses a method for generating a recombinant library by introducing one or more changes within a predetermined region of double-stranded nucleic acid, comprising providing a first primer population and a second primer population, each of the populations having a variable base composition at known positions along the primers, the primers incorporating a class IIS restriction enzyme recognition sequence, being capable of directing change in the nucleic acid sequence and being substantially complementary to the double stranded nucleic acid to permit hybridization thereto. The method additionally comprises hybridizing the first and second primer populations to opposite strands of the double stranded nucleic acid to form a first pair of primer-templates oriented in opposite directions, performing enzymatic inverse polymerase chain reaction to generate at least one linear copy of the double stranded nucleic acid incorporating the change directed by the primers, cutting the double stranded nucleic acid copy with a class IIS restriction enzyme to form a restricted linear nucleic acid molecule containing the change, joining termini of the restricted linear nucleic acid molecule to produce double-stranded circular nucleic acid and introducing the nucleic acid into compatible host cells. A method is additionally provided for generating a recombinant library using wobble-base mutagenesis.

DETDESC:

DETD(49)

Library mutagenesis using a **heterogenous** primer population permits incorporation of a large number of mutations into a population of host cells to generate a recombinant **library**. The resulting mutations are typically introduced into a polynucleotide suitable for cell delivery. The polynucleotide can additionally be adapted for. . . or confer a particular cell phenotype. The incorporation of a large number of mutations into a host population is termed **library** mutagenesis. In general, **libraries** can be prepared and screened for changes in any measurable cell property. Similarly, the transformed or transfected cells containing the. . .

=> d cit ab kwic 2

2. 4,939,666, Jul. 3, 1990, Incremental macromolecule construction methods; Karl D. Hardman, 364/496; 436/86, 89; 935/87 [IMAGE AVAILABLE]

ABSTRACT:
A preferred embodiment of the invention is a method for constructing a polypeptide chain having a substantially predetermined conformation. Preferably a known stable well-mapped polypeptide structure is used as a starting point, and additional peptide units are incrementally added on while maintaining favorable enthalpic and entropic contributions to stability. Preferably a library of oligopeptide blocks is used to provide candidates for the additional peptide units. Preferably the library includes numerous precomputed parameters for each of the blocks, e.g. parameters for estimating energetic effects of varying the conformation parameters.

SUMMARY:

BSUM(169)

For . . . (e.g. the six most common hexoses plus fucose, and/or triplet blocks containing sequences of these) can also be used as **library** blocks. In embodiments where the **library** is allowed to contain **heterogenous** blocks--e.g. where the **library** contains both oligosaccharides and oligopeptides--appropriate penalties should be added into the figure of merit calculations, so that the optimization process will not introduce disfavored heterosequences unnecessarily. Moreover, the transition blocks should also be included in the **library**, if construction across the transition region may be desired. For example, glycoproteins require one of two specific links ("O-links" at. . . those skilled in the art.) Adjacent monosaccharides can have steric hindrance constraints, so that the advantages of storing oligopeptides as **library** blocks are also partly applicable to oligosaccharides (if glycoprotein or polysaccharide construction is desired).

=>

=> logoff

ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y

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